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FIFTH ANNUAL REPORT

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CANCER LABORATORY

OF

New York State Department of Health

conducted at the

GRATWICK RESEARCH LABORATORY
UNIVERSITY OF BUFFALO

For the Year 1903-4

FIFTH ANNUAL REPORT

OF THE WORK OF THE

CANCER LABORATORY

OF THE

NEW YORK STATE DEPARTMENT OF HEALTH

Conducted at the
GRATWICK RESEARCH LABORATORY
University of Buffalo

For the Year 1903-4

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REPORT OF THE DIRECTOR.

BUFFALO, N. Y., *March* 17, 1904.

Dr. DANIEL LEWIS, *Commissioner of Health, Albany, N. Y.:*

Dear Sir.—I have the honor to transmit herewith the annual report of the Cancer Laboratory researches carried on under the auspices and by the aid of the Department of Health of the State of New York and in the Gratwick Laboratory of the University of Buffalo. The past year has not been characterized by any marked discoveries which can be safely announced to the world, but has been marked by steady, earnest and even laborious study along the lines of investigation, all of which are necessary and all of which are lending improvement in the study of cancer, either by chemical or biological means.

During the past year the staff of workers has included the following:

Dr. Harvey R. Gaylord	Pathologist.
Dr. H. G. Matzinger	Bacteriologist.
Dr. G. H. A. Clowes	Chemist.
Dr. Gary N. Calkins	Consulting Biologist.
F. S. Low	Assistant in Photo-chemistry.
W. S. Frisbie	Assistant Chemist.
Alice G. Owen	Assistant.
Clara A. Maclay	Secretary.
F. A. Payne	Janitor.
M. Weber	Laborer.
E. Kempkes	Laborer.

A portion of this time has been spent by Dr. Gaylord in Europe. Inasmuch as he was not working entirely in the interests of the Laboratory his salary was proportionately reduced. He has taken advantage of his presence in Europe to attend regularly the meetings of the Royal German Cancer Committee, of which, with myself, he is a member. He went to Copenhagen in the interests of the Laboratory to investigate the work of Jensen on the transplantation of carcinoma in mice. Professor Jensen was kind enough to give Dr. Gaylord some of his mice, which were brought to the Laboratory and used by Dr. Gaylord for the continuation and elaboration of the transplantation problem. A report from him is transmitted herewith. I also transmit herewith a paper by Prof. Gary Calkins, in which he has paved the way for still further announcements, of whose importance when made there can be no doubt.

One feature with which our study is more and more concerned is the variation of tumors of apparently the same general type, which are found to vary more and more, as do the personal features of different individuals of the same family. So marked is this variation that it becomes often confusing and makes it not only difficult to assign given specimens to their proper positions but even to indicate their relative degree of malignancy. Special attention has been given for the last few months to the study of tumors of the breast, in which Dr. Matzinger has been leader, and along the lines on which important theories have been advanced by Creighton in his remarkable new work entitled " Cancers and Other Tumors of the Breast."

The principal work of the chemical department in the course of the last year has been devoted primarily to the solution of the problem of obtaining immunity against cancer. Horses are being employed to prepare sera for use in incurable cases. Work of such a nature necessarily extends over long periods of time, and it will be impossible to give any final reports on this work in the immediate future. Experiments having a similar object in view are also in progress, making use of cancer mice kindly placed at the disposal of the Laboratory by Dr. Jensen of Copenhagen.

A great part of the time of the Laboratory staff has been devoted to work of a routine nature, several hundred analyses of stomach contents, urine, etc., having been carried out for the purposes of diagnosis. In several cases in which nutrition of patients has been seriously impaired systematic analyses of food and excreta have enabled us to materially improve their condition.

A part of the work undertaken by Dr. Clowes has been the chemical testing of the various staining fluids and materials used in order to better appreciate their reaction upon the cells constituting tumors, a subject on which there is much yet to be learned which will prove of value.

From the papers incorporated in this report and the abstracts of those published in the course of the last year in other journals, it will be seen that a considerable amount of work has been accomplished bearing on the physico-chemical problems of metabolism, the solution of certain micro-chemical problems and work of a serum-therapeutic nature.

The study of cancer everywhere provokes universal interest, evidences of which are seen in the increase of the activity of the medical journals devoted to this topic. A recent number of the British Medical Journal was almost exclusively devoted to the subject. There is now published in Germany, under the auspices of the German commission for the study of cancer, a *Zeitschrift für Krebsforschung* devoted exclusively to the subject, of which three numbers have already appeared. It proves to be a journal of great value. Such widespread activity is manifested in these various ways as to indicate the importance of this study to humanity.

In the last annual report I submitted a paper which had to do with applications, especially of the X-ray, in the treatment of cancer. In this paper I took the stand that it was too early to undertake to report results or do anything but gather material and watch. Now, a year later, I hardly feel justified in making any different statement, since while a large amount of material has been gathered and while men have watched eagerly, reports are so confusing and results so variant that it is still difficult to form a judicial estimate of their actual value. Recurrence has taken place in a few of the instances previously reported as cured and the whole question is still *sub judice*. I have had it in mind to summarize in a similar manner the subject of radiotherapy, but here the same confusion prevails. Opinions regarding this subject are discrepant. It is usually those who favor the use of the treatment, or those who have taken it up as a fad, who write the majority of the articles. Men who have been disappointed in its use or who have never become converted to it generally keep silent, and so their views are not heard.

Treatment of superficial growths by liquid air constitutes a method much more tangible, and, in some instances at least, promising. The conditions surrounding the method, however, make it impracticable.

Herewith is submitted a table, arranged by months, of the deaths occurring from cancer in the State of New York as taken from the Monthly Bulletin of the Department of Health. It shows an increase of about five hundred; that is to say, ten per cent, over the total number the previous year. How this figure can be explained on any basis other than actual increase I can not see. Certainly diagnostic methods have not so far improved within the year as to account for any such augmentation as this. It constitutes a rather startling corroboration of the estimate and even the prophecy contained in my first annual report of five years ago. I do not know what stronger argument could be advanced in favor of public assistance for the study of this disease than these figures furnished by disinterested observers.

TABLE I.

December, 1902	458
January, 1903	467
February	422
March	478
April	453
May	429
June	450
July	463
August	470
September	475
October	456
November	415
Total	5,436
For the year ending December 1, 1902	4,984
Increase	452

Below is submitted in Table II. a statement of the expenses of the Laboratory arranged as heretofore.

TABLE II.

Expenses of Conducting the Laboratory for the Period from October, 1902, to October, 1903.

1902.	Equipment.	Stock and material.	Sundry expense.	Salaries.	Total.
October	$100 81	$58 76	$90 15	$728 33	$978 05
November	97 48	195 95	105 54	762 33	1,161 30
December	40 10	95 91	224 48	809 83	1,170 32
1903.					
January	102 51	102 92	119 31	790 33	1,115 07
February	42 63	193 19	396 07	1,040 32	1,672 21
March	208 79	158 41	180 40	873 66	1,421 26
April	36 38	56 82	145 17	873 66	1,112 03
May	9 12	58 93	155 68	873 66	1,097 39
June	49 78	39 51	399 08	873 66	1,362 03
July	179 66	37 14	326 49	706 99	1,250 28
August	2 50	7 04	123 37	694 99	827 90
September	384 02	23 60	27 44	700 04	1,135 10
	$1,253 78	$1,028 18	$2,293 18	$9,727 80	$14,302 94

Of our appropriation of $15,000 for the year 1902-3, $651.32 was used before October 1, 1902, on account of the change in the fiscal year which made it necessary for the previous appropriation to stretch over twenty months; and we had on hand October 1, 1903, $45.74, which accounts for the full $15,000.

With regard to the needs of the Laboratory for the ensuing year, I can only say that it is contemplated to carry on the work about as previously. The appropriation heretofore, i. e., $15,000 per annum, just enables us to do this work, and I would respectfully urge a continuation of the appropriation of the same amount for the continuation of the work during 1904-5. I have observed no falling off in zeal and interest among those engaged in the work; on the contrary, each one connected with the institution has shown such a degree of interest and faithfulness as to be entitled to much public commendation. As before, I have to thank you again for the unfailing assistance which has been constantly rendered from your department and for the numerous evidences of your own personal interest and appreciation of the work done in Buffalo.

ROSWELL PARK.

THE CELL-INCLUSIONS IN CARCINOMA.

By GARY N. CALKINS, Ph. D., Professor of Invertebrate Zoology, Columbia University, N. Y.; Consulting Biologist State Cancer Laboratory.

It is not my purpose in this report to drag in again the arguments for and against the parasite theory of cancer, nor to dwell upon the lines which such arguments have taken in the past fifteen years. My subject deals rather with the miscellaneous inclusions found in cancer cells and leads me to offer still another contribution to the already crowded literature upon the subject of these much contested structures. I do so not with the hope of saying a conclusive word upon this difficult topic nor of formulating a theory as to the aetiology of cancer, much less of announcing a "new cancer organism," but solely for the purpose of showing how these inclusions may be regarded by a student of cellular biology who has given particular attention to the structures of the cell as they appear in normal tissues and in the lowest animal forms.

It may be pointed out that biologists as a rule have given but scant attention to the cancer inclusions, the majority of them passing over the subject with little thought, or with some evidence of arrogance dismissing without study the characteristic structures as "more like technical artefacts than organisms." There has grown up, however, a great literature on the subject of organisms, especially protozoa, in cancer, most of which, to be candid, is unquestionably based upon a meagre knowledge of these lower forms of life. Assertions and generalizations have been made which not only betoken ignorance of protozoön morphology, but owing to their indefinite nature invite criticism from the biological side on the one hand and scepticism from the pathological side on the other.

The main contention which some writers have held that the inclusions are protozoa has been weakened by false analogies and comparisons and the facility with which such comparisons have been overthrown has discredited the majority of such attempts. On the other hand, a biologist attempting to interpret the varied structures in cancer cells invites the same form of criticism from the pathological side, and basing his conclusions on morphology alone, runs the risk of making equally false and unjustifiable assertions and generalizations. It is with the full conviction of this possibility of error that I offer in the following pages a critical review of the structures which I have independently observed during the time that I have been connected with this Laboratory.

Contrary to what appears to be the usual custom in studying cancer-cell inclusions, I have not examined a large number of tumors in support of any particular hypothesis. On the whole it seemed to me more profitable to study in detail a single case and to be familiar with all of the types of inclusion which that case might present. The advantages of this procedure are: (1) It makes possible the attempt to work out the relations of the different kinds of inclusions, to grade together those which are undoubted degenerations or secretions, and those which may be otherwise interpreted. (2) It presents all of the inclusions under the same conditions of the disease. (3) It permits a deeper analysis in a given time into the structural modifications of the particular type of cancer cells under observation. (4) It gives by reason of this more detailed study a better basis for comparisons with the inclusions in other types of tumor.

Although I have examined many different types of tumor more or less carefully, the one which I have studied in detail is designated as case 300D in the Laboratory records. It was a rapidly growing carcinoma of the breast, operated by Dr. Roswell Park in June, 1903. The report of the pathologist upon it is as follows:

On examination the tumor proves to be a soft solid carcinoma of the breast, in many places showing a distinct cystic character. The larger cystic cavities are lined with multiple layers of large epithelial cells, the nuclei and protoplasm of which stain deeply with haematoxylin-eosin. Karyokinetic figures are fairly frequent. In the smaller nests the membrana propria of the ducts is broken through and nests of epithelial cells can be found in the connective tissue. Many of the canals are filled with solid masses of epithelium. The stroma is well developed and shows a moderate round-celled infiltration. The larger cystic cavities contain a coagulated secretion. In some places the epithelium presents characteristic papillary growths protruding into the lumena of the canals.*

It is the experience of all cytologists that the finer structures of the tissue cell such as centrosomes, spindle fibres, protoplasmic network and the like, can be satisfactorily demonstrated only when the tissue is fixed at once after removal from the body. By such immediate fixation the parts of the cell are set or coagulated by the fixing fluids before they have much time to change. Such changes are indicated in poorly fixed tissues by shrinkage of different parts of the cell and by the impossibility of demonstrating the various structures. Thus the cytoplasm appears shrunken away from the nucleus; the chromatin in the latter disintegrates while the nuclear membrane appears wrinkled and abnormal. In such cells it is practically impossible to demonstrate complete division figures, the spindle-fibres and centrosomes of the mitotic figure being indistinguishable, while the chromosomes are massed together in formless bulk. Conversely a good test of fixation and of stain is the clear demonstration of each of these parts of a dividing cell. If every spindle fibre is clearly defined, if each chromosome stands out sharply and distinctly, and if the centrosomes appear in their proper places as minute deeply-staining points, while in resting cells, if the nuclear membrane appears full and even, with the accompanying cytoplasm free from vacuoles, then the observer will have material from which he may confidently hope to get results.

It is not easy to get this perfect fixation in epithelial cells, but it is far more difficult to get good results in the structural details of the cell-inclusions. Notwithstanding their unicellular nature even protozoa are difficult to fix properly, as any one can testify who has attempted to fix *Amœba proteus* for example, with its pseudopodia extended, yet it can be done by using proper precautions, and it is equally possible to demonstrate structure in the cancer-cell inclusions. In my opinion the so-called " Plimmer's bodies " or " birdseye " inclusions are but poorly fixed bodies, the structures of which are masked because of poor technical methods.

---

*Dr. Gaylord.

Every precaution was used in the treatment of case 300D (cf Figs. 34 and 59). Immediately after removal the tumor was brought in a hot box to the laboratory not 100 feet away, and long before the surgical details in the operating room were concluded, it had been cut into small pieces and distributed among several fixing agents. These included the fluids which have been tested by cytologists and recommended for their power of penetration without distortion. Zenker's fluid, Hermann's fluid, sublimate-acetic, picro-acetic and saturated sublimate were used, the best results being given with the first two. Among the stains used were Heidenhain's iron-hæmatoxylin, Delafield's hæmatoxylin, and Feinberg's stain, all of which gave passable results, but none of these was as satisfactory as the Romanowsky stain of polychrome methylene blue and eosin, nor as the magenta, picric-acid and indigo-carmine stain of Borrel (1901).

A. CRITICAL EXAMINATION OF THE CELL-INCLUSIONS.

The interpretation of a given cell-inclusion in carcinoma can be accurately given only by the genetic history of that inclusion. Hypothesis may follow hypothesis regarding it, but until it is followed out stage by stage the inclusion must remain a puzzle. It is fully as important to get the consecutive steps in the formation of a cell-inclusion as it is to get the series of changes in the life history of some unfamiliar animal, and inclusions in carcinoma will be a source of trouble until this is done for every type found.

Many of the inclusions in carcinoma have an encapsuled appearance due to the presence of a more or less thick membrane separating the inclusion from the cell protoplasm. Others are naked in the protoplasm and, if fixation is imperfect, they are apparently suspended in a vacuole. Secretions, degenerations of portions of the cell, metaplasmic products and the like are naked, and so also are intra-cellular parasites in trophic phases. The great membranes or capsules seen in so many cases are in all probability due to the reactions of the cell protoplasm to foreign bodies and not a portion of such foreign bodies, and the problem of such inclusions may be limited to identification of the contents of the capsule. No such capsules appear in the known cases of intra-cellular parasites, possibly because the protoplasmic reaction which produces the capsule is not called into action, possibly because of some emanation from the parasite which prevents its formation, for it may readily be seen that such a wall would be detrimental to the life-processes of a parasite by shutting off the food supply, walling in waste products and the like. So too the capsule which may form around a foreign body not a parasite; e. g., a blood cell, may cause the disintegration of such a contained cell by similarly keeping out food and keeping in waste products. We may state as a general principle regarding cellular parasites that no living parasite while performing its ordinary vegetative functions within a cell host is separated from its source of food supply by a capsule. A capsule may be formed, however, by the animal parasite itself during its reproductive phases, or the reproductive bodies such as spores may be protected temporarily by such capsules, while after the parasite is dead a wall may be built around it by the cytoplasm of the host cell. This capsule about these carcinoma inclusions then may be used as a convenient morphological element for distinguishing certain histogenetic products within a cell from the metaplasmic products of the cell or from intra-cellular parasites. It may define one type of cell-inclusions, while the absence of a capsule may involve further analysis. If the inclusions are cellular in nature, i. e., provided with nucleus and cytoplasm, they might well be regarded as parasites; if colloidal or uniformly granular, or otherwise specifically characterized, they may be regarded as products of the cell. Obviously, so far as demonstrating the parasitic nature is concerned, the capsule furnishes only negative evidence and the real difficulties in interpretation come in distinguishing products of the cell from non-encapsuled invaders.

Applying these considerations to the inclusions in carcinoma we find that this differential is of value in the interpretation of many of the structures observed. We note the presence of the capsule about cells which, despite their positions within other cells, may be more or less easily identified as leucocytes, or lymphocytes, or other tissue cells which in one way or another have come to be intra-cellular foreign bodies. We also find the capsule about inclusions which are not thus easily recognized, which show no indication of an original blood-cell structure, but by reason of the capsule about them we may conclude that they are derivatives of such invading cells. This conclusion for any specific case is strengthened by reason of more or less definite intermediate stages. On the other hand, there are innumerable inclusions which do not show the capsule and which have an entirely different appearance from the original cell-invaders or their derivatives, and which may be interpreted in some other way. The presence or absence of a capsule thus seems to offer a purely artificial but a convenient way of attacking the manifold structures which have been described again and again as the " cell inclusions in carcinoma."

I. THE ENCAPSULED FORMS.

The thickened rim or capsule about this type of cancer-cell inclusions is a good example of a widely spread protoplasmic reaction. A foreign granule under an oyster's mantle causes local irritation and secretion, resulting in the formation of a pearl. Many insect eggs cause local proliferation of vegetable cells and the formation from those of a thickened coating and other similar phenomena will suggest themselves. While these cases are instances of multicellular reactions to foreign objects, the same kind of reaction may occur when objects are introduced into the protoplasm. A notable example is the thickening of endoplasm about the water in a contractile vacuole in amœba, the condensation resulting in the formation of a capsule similar in nature to the ectoplasm which surrounds the entire animal, and like this brought about by the protoplasmic reaction to the water. It is apparent that the wall of the vacuole in amœba is formed by the compression of the alveoli which make up the structure of the protoplasm of the amœba's body, for the diameter of these alveoli decreases from the endoplasm towards the rim of the vacuole where owing to the density the separate alveoli can no longer be made out.

The conditions of the capsule about a carcinoma cell-inclusion are very similar to those of the thickened protoplasm about the contractile vacuole in amœba. There is the same gradation in density from the very dense inner wall turned towards the inclusion to the outer, normal, cytoplasm of the cell. Ribbert ('91) called attention to the fact that there appears to be a double contour in this membrane and he interpreted it as a thickening in the surrounding protoplasm, but accounted for it by the supposition that when one cell becomes invaginated into another the cell membrane of the invading cell would appear like a capsule. The capsule according to this interpretation therefore would be a part of the invading cell. Other observers interpret the capsule as a condensation of the protoplasm in the invaded cell. This was Sjöbring's ('90) interpretation and it was accepted and confirmed by Sawtschenko ('95), by Ruffer and Walker ('93) and by many others. Sawtschenko observed that the protoplasm of the invaded cell becomes more and more dense as the membrane is approached until in the membrane itself the protoplasm is homogeneous and clearly defined.

The explanation of these capsules is not clearly apparent unless they may be regarded as the result of a protoplasmic reaction to a certain type of stimulus. It is difficult to see how the invagination of one cell by another can give rise to all of the structures provided with capsules in the cancer cells. Ruffer and Walker ('93) express the view that the invaginated cells, due to rapid growth and pressure in a small space, may take the form of a cigarette within its holder. A section of such a combination would show two circles within a circle. In the case of cells, one of which is invaginated within another, we might similarly find an outer membrane of the invaginated cell and two inner membranes, one within the other, belonging to invaginated cell and invading cell respectively, and we could imagine that the nucleus of the invading cell might be pushed to one side as shown in Fig. 20 while some of the nuclear structures might even be interpreted in this way. The difficulty comes, however, in interpreting the degenerated structures within these capsules. If nothing but pressure induces this invasion, what reason is there for the degeneration of the invader and not of the invaded?

The natural supposition would be that it is the latter with its displaced and compressed nucleus in which we should look for ill effects and not in the former. Nor does this theory account for the thickening of the membrane in what may be properly interpreted as inclusions which have been in the invaded cell for some time, nor does it account for the graded density of the plasm which Sawtschenko correctly recognized. While it is not improbable that some of the inclusions found in epithelial cells in carcinoma are invaginations, and that the membranes in such cells may appear like thin capsules, there is reason to believe that this interpretation is too limited to account for all the inclusions with capsules of varying thickness.

Many of the cell-inclusions have no evidence of epithelial cell structure and may be recognized without much difficulty as leucocytes or lymphocytes, etc., and the capsules about them are well-defined and conspicuous (Figs. 2, 3, 5, 19, 20). The possibility of leucocyte invasion is admitted by almost every observer, and it is taken for granted by many that degenerative change of these invaders may give rise to many of the types of cancer-cell inclusions. Sawtschenko ('95) gave a very good review of the possibilities of such changes and described some of the modifications which follow upon invasion. Ribbert attempted to harmonize his theory of invaginating cells with this view by admitting that invagination in some cases might go so far that the invaded cell entirely closes over the invader, and he described the subsequent changes that the imprisoned cell might undergo; most of these, however, apply to the changes which leucocytes or other blood cells undergo and not to invaginating epithelial cells. Klebs ('93) undertook to explain the subsequent changes of invading blood cells on the supposition that they are digested by the epithelial cells which harbor them, and he maintained that many of the structures which other investigators took to be parasites were in reality only stages in the digestion. Without digressing too far to consider this theory, which begs the question of intra-cellular digestion on the part of epithelial cells, it is certainly unquestionable that any cell found within the protoplasm of another cell will, unless it is a parasite and adapted for this particular environment, be unable to continue its own living activities and will die, especially if prevented by a capsule from absorbing food or disposing of waste matters. Kleb's view that the cell is digested seems hardly necessary to account for the disintegration and degeneration which must follow as a natural result from the process of encystment. Whatever the agents are that bring about the degeneration it seems to be a recognized fact that such modifications of leucocytes and lymphocytes take place, and that the products are stored for a longer or shorter time in the protoplasm of the invaded cell.

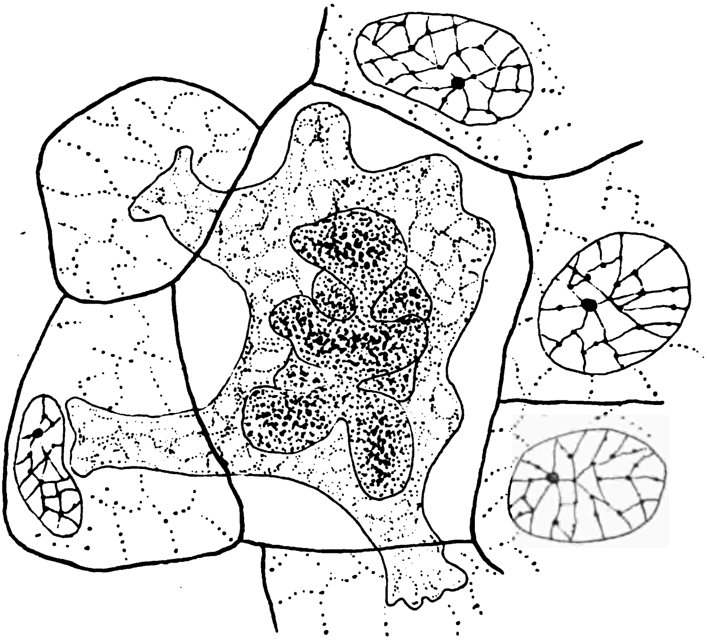
There is little doubt that a structure like that pictured in Figure 2, is a blood cell included in an epithelial cell, and incased within a capsular membrane derived from the protoplasm of the invaded cell. In these cases the original nature of the invading cells is retained and shows the specific character of the inclusion. The capsules are equally characteristic and are very different from the original cell membrane of leucocyte or lymphocyte.

With inclusions of the type represented in Figure 7, the specific nature can not be thus easily recognized and nothing remains except the capsule to aid the identification. This, however, is sufficiently characteristic to warrant the identification as a degenerated blood cell, and the conclusion is strengthened by the study of what are undoubtedly intermediate stages in the disintegration and degeneration of the recognizable blood cells. Here again the capsule plays an important part, for in cases which may be interpreted as later phases of degeneration the capsule appears thicker and denser than in the earlier phases (compare Figures 3 and 6). As Klebs pointed out the first changes which an invading cell undergoes are in the cytoplasm. This becomes "stringy" and incomplete through the appearance of great vacuoles (Figure 19) or it may be drawn out into strands like pseudopodia (Figures 21 and 23) or packed tightly about the yet unchanged nucleus (Figure 2). At a later stage the nucleus becomes modified; its reticular structure disappears, and it becomes compressed into a more or less solid spherical mass of chromatin (Figures 2, 5 and 21). A complete series of these modifications might be obtained without much difficulty from a single cancer, and a few of the characteristic forms assumed are shown in Figures 1 to 7, 16, 17, 19-24, 26 and 38. One important feature is the fact that the cytoplasm of the invading cell does not disappear; that is, it is not digested as Klebs assumed but persists and gives to the body within the capsule an appearance strikingly similar to an amœba, or to a spore of Plasmodiophora within its cyst. The nucleus in such forms varies considerably in size (cf. Figures 21, 22 and 23) and in some cases it apparently breaks up into granules as in Figure 6. Usually it is very much reduced and occasionally appears as a mere fragment of nuclear material within a mass of protoplasm which has no longer even the semblance of a blood cell. Finally this type of inclusion may be traced to structures like those represented by Figures 7 and 24, where hyaline degeneration has resulted in the formation of one or several structureless albuminoid masses with little or no resemblance to the former blood cell.

The earlier writers and observers were inclined to regard these various structures as sporozoa, especially coccidia. This was the interpretation put upon them by Strœbe, by Podwyssotzky and Sawtschenko, by Foa, by Metschnikoff and many others. Some, on the other hand, like Ribbert or Klebs, regarded them as products of degeneration. In addition to these there were still others who were uncertain as to the true significance of these structures, and did not attempt to place them. Among these was Soudakewitch ('92), who declined to place the inclusions which he was unable to identify as degeneration products in any group of animals or plants. Less conservative were Heukolom and Sjöbring, who abandoned the notion of coccidia in favor of the microsporidia, claiming that the inclusions are much more like the organism which causes pèbrine in silkworms than like coccidia. Part of Sjöbring's objection to the so-called coccidia of other observers was correctly based upon the fact that the apparent membrane around the supposed organism increases in thickness, and this he could not harmonize with a capsule formed as a cyst, a structure which, in other sporozoa, when once formed does not increase in thickness.

In reviewing the early work on these cancer-cell inclusions one is struck by the fact that the various degenerating cells of this type have played a most important part in filling in the stages in the "life-histories" of supposed parasites. The majority of observers have described forms of all sizes and shapes varying, as a rule, from 3μ to 40μ, the larger ones with solid and homogenous nuclei being almost invariably regarded as the adult form, and as these are usually found in many stages of disintegration, or as the capsule is frequently found with many structures within it, they were not infrequently interpreted as reproductive bodies ("the sporocysts" of many of the early observers cf. Fig. 7). In one case at least (Korotneff, '92) there appears to have been a confusion of large extra-cellular colloidal masses with these cell degenerations and with the minute bodies which subsequently became known as Plimmer's bodies or the "birdseye" inclusions. Korotneff's supposed organism, which he named *Rhopalocephalus carcinomatosus,* was regarded by him as a connecting link between the coccidia and the gregarines, and was endowed by him with characters of zoological as well as pathological importance. This called out immediate criticism from zoologists and pathologists alike, and the result is that Korotneff's "organism" enjoys the empty honor of a mere historical incident.

Before leaving this type of cancer-cell inclusions we must consider another very questionable organism, which, like Korotneff's Rhopalocephalus, is relegated to the waste pile of zoological genera and species. I refer to the remarkable structures which Eisen interpreted as living, independent amœboid organisms, causing carcinoma. I have reproduced one of these structures from Eisen's paper, and have searched for them in the tissues of the cancer which I have studied. It is difficult to prove that the bizarre cell-structures which I have found are identical with those described by Eisen, but as he maintained that the organisms were very common in cancers of this type I think there is no doubt that I have frequently seen what he means by his *Cancriamœba macroglossa* (compare text-figure and my plate II, Fig. 36). A characteristic feature is the "projectile snout" upon which Eisen bases the specific name, and which he regards as a pseudopodium with the specific function of food-getting. This snout, as he believed, presses into an epithelial cell like the suctorial tentacle of an acinetan and gradually digests the protoplasm of the invaded cell. The nucleus of the parasite is described as polymorphous and is frequently seen in the process of segmentation or fragmentation a process which precedes sporulation.



Copy of one of Eisen's figures of "cancriamoeba macroglossa" to show relative size of cells, "snout" etc.

Without going into details upon the subject of Eisen's interpretation of cancerous growths, and their relations to the supposed parasites, I may give a few criticisms of the supposed amœba, criticisms which are meant seriously since it is quite evident that Eisen's work was done in that spirit. The first point that strikes a student of the protozoa is that the protoplasm of the supposed parasite and that of the epithelial cells is almost identical in structure; the characteristic alveoli of amœba-protoplasm are conspicuously absent. As it is a cytophagous form food vacuoles in which the absorbed protoplasm from the host cells should be digested are always absent; there is no sign of ectoplasmic differentiation, the membrane around the supposed amœba is exactly the same as the membrane of an epithelial cell; contractile vacuoles, while not absolutely necessary for amœbæ, are nevertheless usually present, are entirely wanting in the form under consideration. (Note that in Schaudinn's Leydenia ('96) found in the fluids of peritoneal tumors a contractile vacuole is present.) The nucleus which Eisen fills in with structural detail is entirely different from the nuclei of any species of amœba with which I am familiar, for the characteristic marks of a rhizopod nucleus are large granular masses of chromatin or "chromatin reservoirs" suspended apparently in a nuclear fluid and surrounded by a very definite nuclear membrane. In the cells which I have found in carcinoma and which I regard as the same things that Eisen has described, the nucleus is exactly of the type as those of the surrounding epithelial cells, with the exception that it has lost its definite outline and appears hypertrophied, indented and polymorphic. The chromatin content of the nucleus is very different from that of a rhizopod, for chromatin and limin reticula are readily discernable.

Without attempting to demonstrate the histological significance of this type of structure, which appears to me simply a deformed cell through pressure in the epithelial plug, and undergoing hypertrophic degeneration, I may classify this supposed parasite as another case of mistaken identity which would have been avoided if the author had had a more thorough knowledge of the rhizopod organisms.

Quite a novel interpretation of this type of inclusion was given by Sawtschenko in his later and more important work in 1895. His observations were based upon well hardened and well stained material, and his conclusions regarding the larger cell-inclusions were similar in nature to those of Ribbert and Klebs, who saw in them only the remains of invading cells. Sawtschenko interpreted these inclusions as slimy or colloidal degeneration of cells or parts of cells, but he believed the material so formed to be the food-medium of real parasites of minute size and of varying form. The great majority of inclusions which had been described previously to his work he regarded as merely the scene of activity of the active, living inclusion, which in his earlier paper ('92) he had described as the "tadpole" form. In the later paper these forms were described as present in many different phases and his descriptions are so careful and his drawings so accurate that many of them might well be given to-day as a faithful representation of what is undoubtedly the most important and most interesting type of the cancer-cell inclusions. These inclusions, generally known to-day as Plimmer's bodies, or by von Leyden's term "birdseye" inclusions, have been the subject of contention between the recent advocates of the parasite hypothesis and their opponents. They are the astrospheres and centrosomes of Borrel ('01), the "cancer-parasites" of Bosc ('98), the "Plasmodiophora-like" bodies of Gaylord, the "Histosporidium carcinomatosum" of Feinberg, and the "intra-cellular secretions" of Nósske and Greenough, the Chytridae of Behla as well as the yeast-cell types of San Felice and others; in short, the controversy over the cancer-cell inclusions has been sifted down from the larger structures, which are generally recognized as degeneration products, to these minute inclusions first accurately described by Sawtschenko. The general form of the minute inclusions is that of the familiar Plimmer's body, a vacuole with an inclusion, which with inadequate hardening and staining methods appears homogeneous and usually without structure, thus leading to the misconception of the wall of the vacuole as the cell body and the contents as the nucleus of the supposed parasite. Sawtschenko maintained that the thickened capsule has nothing to do with the parasite save the limitation of the immediate environment, and interpreted the capsule inclusion as cell body plus nucleus. There seems to be little doubt that Sawtschenko confused some stages of degenerating blood cells, especially those forms with thickened capsule, with inclusions like the "birdseye" inclusions which should not be thus interpreted. Many of the cell inclusions of this type were observed by him to have no apparent structure, to be hyaline, and of the nature of colloidal degenerations; in short, the end stages of degeneration, and he suggested that such structures might be the degenerated remains of the parasites themselves and a condition to be expected if the inclusions are parasites, some of which might well be expected to succumb to the reactions of the epithelial cells. This keen observer therefore not only did much to clear the field of the speculative rubbish that had accumulated, but paved the way for future interpretations of the structures which are not to be interpreted as degenerating cells and over which the controversy of a cancer parasite must be fought. Before considering this type of inclusions more carefully it will be well to take up from a general point of view the inclusions which may be grouped together as the non-encapsuled inclusions.

2. NON-ENCAPSULED CELL-INCLUSIONS.

Among the inclusions of this type there are many possibilities. We may include under this heading the various oil or fatty products of the cell, local degenerations in cytoplasm or nucleus, metamorphosing products of nuclear origin, and metaplasmic modifications of various kinds as well as any intra-cellular parasites that may be present. The great difficulty obviously is to distinguish one from the other and any possible parasite from all the rest. On first thought this distinction between a possible parasite and products of the cell itself would appear easy to make on the ground that a parasite must be a complete cell with definite body and definite nucleus, whereas a cell product would be homogeneous and structureless. Unfortunately this is not true. Parasites may be present in which the nucleus is not a clearly defined structure of definite form and size but like the nuclear material of many primitive forms, such as bacteria and flagellates, may be distributed in the form of separated granules. On the other hand, normal structures of the cell may be present which, like the yolk nucleus of many egg cells, or the "Nebenkern" of many early sperm cells, or the "Chromidien" of many protozoa, are nuclear in origin and stain like chromatin, although in the cytoplasm. These are the things in short over which the controversy of the parasite in cancer is being waged and it must be admitted that the advocates of the parasite theory have much to contend with. Some of the inclusions, however, are difficult to interpret except on the basis of foreign organisms, and here we come to the necessity of discriminating between the structures which Behla has aptly termed the X-bodies, and the products of degenerating and invading cells. Let us first examine the other non-encapsuled cell-inclusions such as secretions, degenerations and the like.

A great many forms of cancer-cell inclusions appear homogeneous and without evidence of cellular structure, and might well be explained as degenerations of an albuminous nature. These are sometimes found in the cytoplasm and sometimes in the nuclei of epithelial cells, but wherever seen can be readily distinguished from the other inclusions. The possibility of inclusions being merely colloidal degenerations was pointed out by Soudakewitch in 1892, but the type was not emphasized until more recent times when Sawtschenko, Pianese, Fabre-Domergue, Hansemann, Nösske, Greenough and others have called particular attention to this possibility in connection with the contested inclusions.

It was pointed out by Sawtschenko and Pianese in particular that these colloidal matters may be formed from degenerating portions of the cell or nucleus, and figures have been given by each of these observers indicating the consecutive changes which the structures in question may take. The various *intra-nuclear* parasites of Thoma ('89), of Sjöbring ('90), of Soudakewitch ('92) and Ruffer ('93) were regarded as degeneration products of this type and the formation of chromatin masses of spherical form, vacuolization and changes in color reactions of the masses were described in detail. Such degenerations are common in the cancer which I have studied, and the figures of Sawtschenko and Pianese might have been duplicated many times over. They have also been found in the nuclei of skin cells in smallpox and in diphtheria and similar vacuolization and change in color reaction noted. They are, therefore, inclusions which all students of animal tissues are familiar with, and I may pass them over with the description of one or two. At the outset, as it were, of degeneration, these masses are homogeneous and stain uniformly. (Plate 3, Fig. 39.) Later the central part becomes less sensitive to the chromatin or basic dyes and gradually stains with the acid dyes; the peripheral portion of the mass, however, retains its staining capacity for a longer period and a somewhat shell- or capsule-like structure results. Still later the central portion becomes more disintegrated through vacuolization and may ultimately disappear altogether, leaving a hollow shell or a vacuolated or clathrate structure to mark its place inside the nucleus (Figs. 39-42).

In the cytoplasm similar homogeneous masses may also occur through the degeneration of a portion of the cytoplasm or by the degeneration of an invading cell, or possibly by "intra-cellular secretions." Such changes are known as normal processes in many types of cells which can not be correlated in any way with pathological conditions. Thus in the early egg cells of all types of animals where this subject has been investigated the first appearance of the later yolk material is in the vicinity of the cell-nucleus where it lies like a cap about the nuclear membrane. As the "yolk-nucleus" it has been described by many observers, and in all cases the staining reactions indicate that it is of nuclear origin. With growth of the egg this material becomes scattered throughout the cell body and changes in staining reactions. It ultimately becomes transformed into the comparatively large albuminous "yolk-plates" of the mature egg-cell. In secreting cells of many kinds similar nuclear extravasations have been frequently studied, and the process of chemical metamorphosis has been followed through the many grades of the nucleo-albumens from nucleins on the one hand to clear albumens on the other. In the secreting epithelial gland cells, especially those of the breast, it is to be expected that, if the same origin of the secretion is true, there should be masses of more or less albuminous matter stored in the cell body, and that this matter might show undeniable chromatin staining reactions. It would be expected that such masses might even take a uniform nuclear stain indicating a comparatively high percentage of acid content, or an equally uniform cytoplasmic stain indicating a high and predominating albuminous content, while it is equally to be expected that intermediate grades between the two should be frequently met with. Such inclusions are, indeed, common, and I think there can be little doubt that many of the structures which have been described as sporulating forms of coccidia or large gregarines are to be explained upon this supposition. Even the tadpole forms which Sawtschenko described in his earlier paper ('92) bear the evidences of this mode of origin. This type of structure may not be due entirely to secretions, in the epithelial cells with which we are dealing it may indeed be rare, but similar degeneration may take place in the nuclear material of invading cells which are undergoing disintegration. Thus we may account for structures like those represented in Figure 25, where peculiarly constructed bodies recalling Sawtschenko's "tadpole" forms are distributed about the interior of what appears to be the space occupied by an invading cell in aborted mitosis. This type has been interpreted quite often as a sporulating stage of some organism; e. g., by Soudakewitch, Foa, Metschnikoff, Sjöbring and others, an interpretation which Feinberg, without justification, modifies by considering it the "pan-sporoblast" stage of his microsporidian. In the figure referred to above, some of the contained bodies have a nearly uniform nuclear stain, others a uniform cytoplasmic stain, while in still others part stains with the one, part with the other. The forms assumed by the stained portion are frequently tadpole-like and perfectly homogeneous, and are not sharply demarcated from the remainder of the body. It is to be noted that all of this type of structure are characterized by the fact that the parts which stain with chromatin dyes are on the periphery, and in this respect they simulate the intra-nuclear degenerations described above. Ultimately this type of products of the cell take a uniform cytoplasmic stain and have an absolutely homogeneous appearance.

Still another form of inclusion is frequently found which evidently belongs to this type of colloidal products. These are usually much more fluid than those previously described and give the appearance of oils or fats in the cells. Pianese ('96) has shown the modifications which these inclusions may have, and it is possible to duplicate many of the forms pictured by him in the inclusions observed in case 300D. They are usually inclosed in an irregular area of the cell devoid of capsule-like thickening. The individual globules are of irregular size, probably, as Pianese suggests, due to the fusion of two or more. Figure 27 shows such a type and represents the apparent fusion of a smaller with a larger globule.

3. THE X-BODY.

The so-called X-body of Behla does not find a satisfactory explanation under any of the interpretations of degeneration, secretion, or metamorphosis, and in the cancer literature it has been gradually sifted out from the remaining cell inclusions as a form found only in cancer cells. It is the one type of inclusion which is now generally recognized by friends and enemies of the parasite hypothesis, as the strongest morphological evidence of that theory. It is found in many different phases in the cancer cells of carcinoma, and is the only inclusion in such cells that I have found which in any way justifies the belief on morphological grounds in a possible organism of cancer.

Before giving a review of the previous interpretations of this type of cell-inclusions I would like to describe some of the different forms which it assumes in the cancer which I have been studying. These different forms are so varied and comprise so many different features that at first glance they might be regarded as specifically different things. It is possible, however, to so arrange them that they form a fairly clear and logical set of morphological changes which, if one were so inclined, might be interpreted as growth and reproductive phases of an organism belonging to the unicellular forms. I do not wish to have my meaning misinterpreted in this connection, for there is no positive evidence of a parasite, no sequence in the tissues, and no way to tell, save by possible analogy, that any one stage precedes or follows any other, and to describe in sequence form is partly a matter of convenience, partly to show that such an arrangement is possible, but there is no intention on my part to claim that this sequence in any way establishes the parasitic nature of the inclusions.

In a great many forms of parasitic protozoa the young parasite begins its life as a minute homogeneous spore-like organism with no trace of structure. This is the case in the vaccine organism or young cytoplasmic parasite of the smallpox organism, and the spores of some of the microsporidia which are too small to show the characteristic microspore structures. So in the cancer cells there are minute bodies scarcely a micron in diameter which stain intensely with the specific chromatin dyes (methylene blue, magenta, etc.). These may be the same thing as the so-called Russell's bodies, although I have not observed that they have the characteristic fuchsine reaction which is claimed for these structures and they are much smaller than the forms usually described. They are perfectly homogeneous, sometimes surrounded by a minute vacuole, again with no space about them (Figures 9-10). They are the smallest inclusions that I have seen in cancer cells (less than one micron in diameter), but it is not impossible that still more minute bodies precede these and that more careful technical methods might demonstrate some structure which is certainly not obvious in the preparations as usually made. In slightly larger bodies, equally destitute of membrane or capsule, those for example measuring from 1.5 to 2 microns, there is evidence of differentiation, a minute unstained portion appearing in the body of the inclusion (Figure 10). If we were following the history of an unquestionable organism we should say that the stage following upon the one just described would show a further differentiation of this homogeneous material and that, like a spermatozoon, a portion of the original chromatin-staining substance remains as a nucleus while a peripheral portion forms a more or less clearly defined cytoplasmic or body portion. If this interpretation of these homogeneous bodies were true and if the development proceed as indicated, then the typical Plimmer's bodies might be explained as the immature organism arising from this spore-like germ. It has a definite cell body and a definite cell nucleus, and lies in a characteristic vacuole which always occurs about a foreign body in living protoplasm. In Figure 11 there are two of these forms lying side by side, each is a perfect cell and each is in the form of a minute sphere, measuring 2 and 2.5 microns in diameter. In these particular figures the body of the cell is not drawn out into processes connecting the inclusion with the walls of its vacuole, and this is the usual condition pictured for a typical Plimmer's body (Fig. 8). In the great majority of cases, however, in the preparations which I have studied the inclusion is in connection with the walls of its vacuole by protoplasmic processes which are strikingly like pseudopodia of a myxospore rhizopod-like sporozoon (e. g., Lymphosporidium). The minute bodies are devoid of ectoplasmic modifications and are homogeneous and granular in structure, more dense, however, than the surrounding cytoplasm of the epithelial cell. Occasionally a vacuole is seen in the cytoplasmic portion (Figure 14) but it is not common.

The chromatin-like granule in the center of these small inclusions is perfectly distinct, sharply outlined, and gives an entirely different appearance from the material stained with the chromatin dyes in the types of inclusion described as colloidal degenerations or secretions. It is not a residual color due to the incomplete extraction of the chromatin stain as is the case frequently with colloidal masses or oil-like inclusions, but is a chemically different substance with as definite an outline as a large chromatin granule in the nucleus. This clear definition is retained in all types of the inclusion; sometimes, however, the single granules are replaced by groups of equally distinct granules which may be collected in one group or distributed throughout the small body. With the Romanowsky stain these take the methylene blue, while the minute cell-body takes the eosin; with the Borrel combination the central granule remains red like the chromatin of the epithelial cells, while the cell-body stains green (Figures 12, 14-15, 19, 29, 31, 32, 33, 44-46, 48-51).

The groups of granules are not inconsistent with multiplicative changes in many forms of unicellular organisms. In the rhizopods especially, the nuclear bodies break up into granules by a process called fragmentation; these separate and become the individual nuclei of reproductive bodies. It is therefore a widespread phenomenon of the nucleus and a well-recognized process of division of nuclear material without any of the formalities of mitosis. It sometimes occurs in cases where mitosis is also present, as in *amœba*, where the nuclei divide repeatedly by mitosis until about one hundred are present in the cell, then the chromatin granules in each of the multiple nuclei breaks up spontaneously into small fragments, these escape by rupture of the nuclear membrane, and each fragment forms the nucleus of a spore. These instances serve to show that nuclear fragmentation without a formal process of division is to be expected in the type of organisms to which this cancer cell-inclusion, if it is an organism, may possibly belong. In the small nuclear granule of our form there are no division figures to indicate a mitotic process such as found in the dividing Plasmodiophora for example, but the granules are often seen in an elongated or irregular form, which might be expected in a process of fragmentation. The nucleus-like part of these inclusions is sometimes double, sometimes multiple, but always granular and not homogeneous or diffuse as in colloidal degeneration. The granules are frequently exquisitely minute, so small indeed as to baffle attempt at measurement; the nearest one can get to their size is the general statement that they measure the fraction of a micron, sometimes a large fraction, sometimes small. Whatever the size, however, they are invariably sharply outlined and clearly differentiated. There is never a nuclear membrane, the conditions being similar to the nuclear structures of bacteria or to the protozoon Tetramitus, or Lymphosporidium, or Cytoryctes variolae. Just as in these forms or as in the free non-parasitic types of rhizopods like foraminifera or like the amœba mentioned above the nuclear condition might well justify one in describing this type of inclusion in the cancer cells as a form with its nuclear material in the condition of numerous granular fragments indicating an approaching reproductive process.

It is in this condition of the inclusion, a condition which were we describing a free-living organism might be interpreted as the adult phase, that I have found some of the most interesting and most significant types of the cancer bodies. These are inclusions which give unmistakable evidence of independent protoplasmic movement within the host cells. The most impressive instances of this movement are those cases where the inclusion has been caught as if in the act of migrating from one epithelial cell into another (Figures 13 and 30). In these cases the nuclear material is sometimes in the form of a collection of granules grouped together in a single heap; again the granules are relatively few in number and widely distributed. In Figure 30 the amœboid form is in the act of leaving an intra-vacuolar position in one cell and appears to be penetrating the unaltered protoplasm of an adjacent cell; in Figure 13 a similar condition is pictured, but the connecting strand of material is so thin and delicate that we have the appearance of an indirect division of the inclusion. Indeed this particular case gives the only evidence that I have seen which might be interpreted as a process of division of the inclusion; the chromatin granules are equally distributed and the body is in equal parts. A figure like this, if observed in a free living form like amœba or actinophrys, etc., would be an unquestionable division figure. I have watched the entire process in a minute free form belonging to the genus nuclearia, have seen the pseudopodia drawn in, the appearance of the dividing furrow, the drawing out of the connecting strand of protoplasm and the final break at the center; just prior to the final break the appearance of the two daughter-organisms was strikingly like Figure 13 of the cancer-cell inclusion. It is conceivable that the structure here represented is merely a fragment of a leucocyte and I would be wary of calling it an organism until this possibility is shown to be unfounded.

Still other forms of the inclusions of this type are found in which there is an increase in the number of nuclear granules, each one being separated from all of the rest as in Figures 45, 46, 49 and 50, and occasionally I have found inclusions in which the material is broken up into fragments, each with a chromatin granule in it (Figure 47).

Not only do these inclusions appear in the cell-bodies of the epithelial cells, but they are also occasionally found in the nucleus where they appear to undergo a nuclear metamorphosis similar to that of the cytoplasmic forms. The vacuoles in this case are bounded by chromatin massed to form an apparent membrane which stains with the characteristic nuclear dyes. Several of these intra-nuclear bodies may be traced to metamorphosing chromatin of the nucleus, and there is little or no evidence that they arise by growth of an intra-nuclear spore-like germ. In one case (Figure 28) there are several homogeneous bodies just outside of the nuclear membrane, while two similar bodies are inside of the nucleus and are contained in a vesicle with a delicate wall. Other intra-nuclear forms have the same structure as the majority of the cytoplasmic inclusions, a central or excentric nuclear spot and a differently staining protoplasmic body. In many cases the inclusion appears to be either entering or leaving the nucleus, for the membrane of the latter is deeply indented and the inclusion is partly in and partly outside (Figures 12, 18, 52).

It is quite possible that all of the intra-nuclear forms of the X-body are merely stages in the metamorphosis of the nucleolus as described by Pianese (cf. p. 24). This would explain the chromatin spot and the membrane of chromatin. It might also be argued that the cytoplasmic forms of the X-body are merely the extruded remains of such degenerated nucleoli. The close connection with the nucleus in cases like that pictured in these figures might be cited in support of this theory, but there are so many cases of multiple cytoplasmic bodies, and so many cases where cytoplasmic bodies and nucleoli are both present that the evidence for the theory is outweighed by the evidence against it, while no such theory can account for bodies like those shown in Figures 13, 30, 52, etc.

The large inclusions which had been described up to the time of Sawtschenko, and which have been described by numerous observers since then, were interpreted by him as colloidal degenerations of cells or parts of cells, and a slimy medium was thus formed in which the real cancer-cell parasites found a good feeding ground, and in which they reproduced by the formation of spores of a general sickle-form. The usual inclusions which he described were only once found within the nucleus, the other intra-nuclear bodies which Sjöbring, Soudakewitch and others had laid so much stress on he regarded as degeneration products of the chromatin. His parasites were thought to be more like hæmatozoa than like any other parasitic form and to have a history somewhat similar to the life-stages of the malaria organisms. A nuclear granule was pictured in some forms, a group of such granules in others, while pseudopodial processes formed an irregular peripheral part of the inclusion. The membrane when formed was shown to be a condensation of the surrounding protoplasm of the epithelial cell and no part whatsoever of the organism. It may be seen then that Sawtschenko's observations as to the morphology of the parasite agree very well with the descriptions which I have here given of the

X-body in carcinoma. His material was taken from different kinds of tumors among which were several cases of carcinoma.

There have been many attempts to show that Sawtschenko's "organisms" are products of degeneration, or secretions, or other parts of the normal epithelial cell, but these attempted explanations do not carry conviction, and the failure to find similar things in other diseases, or similarly modified portions of cells in other tissues, goes a long way against such explanation. For the most part these explanations are based upon the possibility of degeneration, or upon some other fanciful change which the cancer cells might mysteriously undergo under the stimulus of unknown toxines or influences of the disease. There is absolutely no doubt that the cancer cells contain inclusions which can be interpreted as degenerations, others as secretions and so on, but in addition to these there are others which can not be disposed of in this way, and these inclusions which Sawtschenko described for the first time, are the forms which remain to be interpreted. An examination of the various attempts to explain them shows the inadequacy of the different interpretations thus far given. Among such attempts we may consider only (1) the astrosphere theory of Borrel; (2) the secretion theory of Nösske and Greenough; (3) the nuclear fragment and chromosome theory of Cornil, Hansemann, Pianese and others.

Borrel's hypothesis has not received much support, nor am I aware that it has been submitted to searching criticism, most observers who deal with the cancer-cell inclusions being content with a simple denial of the possibility. In the early germ cells of the testicles of different animals, especially in many of the vertebrates, the primordial germ cells (spermatogonia) and the maturing germ cells (spermatocytes) have large characteristic structures consisting, as a rule, of several parts, and which are known as the "centrosome" and "sphere" or, all of the structures together, as the "astrosphere." The material of the sphere gives rise in part to the substance of the spindle figure and, when these are present, to the rays passing into the cytoplasm. The centrosomes may in turn have a still smaller internal differentiation the "centriole" and it together with the other sphere substance called the "archoplasm," is supposed to be the center of kinetic activity in the cell. These highly complicated astrospheres are by no means universal in cells and are not easily demonstrable save in germ cells where division is frequent. In epithelial cells they are difficult to make out, although Ballowitz, Heidenheim and Zimmerman have described them in different kinds of epithelium. When present in the cell these spheres are not difficult to stain and are particularly clear and demonstrable when the cells are in active mitosis. At such periods of cellular activity the centrosome and sphere may be seen at the spindle poles, or at the periphery of the daughter nuclei, preparing for the next mitosis. In the resting cells they are conspicuous only in the germ cells or in the spinal ganglion cells of certain animals (dogs, cats, frogs, etc., Rohde). Here they are inclusions with a central spot and an irregular periphery rather sharply marked off from the surrounding cytoplasm but not separated from it by a capsule-like membrane. These astrospheres are the structures which Borrel would compare with the X-body in carcinoma cells, on what justifiable grounds it is difficult to see. Against this view it has been urged that if such structures are normal they would appear in all of the cells while their number would not be increased to twenty or more in any given cell as is sometimes known to happen with the X-body. It seems to me equally plausible to hold the view which Rohde ('03) has recently advanced, that astrospheres in the spinal-ganglia cells are parasites. Both Borrel and Rohde show that the supposed centrosomes divide or else undergo multiple fragmentation to form a group of centers (microcenter) inside of the archoplasmic mass. One (Borrel) interprets the similar division of the central spot of the X-body as equivalent to the fragmentation of the centrosome and regards the central spots as centrosomes. The other (Rohde) holds that the several divisions of the true centrosomes correspond to spores of a parasite. To the former it proves the centrosome nature, to the latter the parasite nature of the central body. To Borrel each part of the cancer-cell inclusion, like the divisions of a centrosome, has the power to form a new centrosome, but each of the divisions of the centrosome forms a new parasite, according to Rohde. To the pathologist the inclusion is a centrosome; to the cytologist, the centrosome is a parasite, and we are justified, I believe, in questioning the advisability of the one on this evidence to enter the field of normal cytology, or of the other to enter the domains of pathology. Borrel's contention can not hold, for the structure in question not only does not have the same history as a centrosome and sphere, but it has no evidence of the structure of this normal part of certain cells, nor is it found at the spindle poles during mitosis, nor at the periphery of the daughter nuclei after mitosis (see Figures 34 and 59). I know of no centrosome or sphere in any normal cell in which there is a like structure, and in cells where astrospheres are formed in large numbers through artificial means, e. g., treatment with magnesium chloride, and which therefore represent abnormal astrospheres of the cell, there is no trace of these peculiar differentiations which are found in the cancer-cell inclusions. In these the artificially created centrosomes and spheres are merely collections of cytoplasmic granules arranged in radiating lines and with a large central granule as centrosome (see Wilson, '02). Borrel's hypothesis then does not stand the test of comparison even with the centrosomes in the spermatocytes of the guinea pig as worked out by Meves, which he takes as his type for comparison, while Rohde's interpretation of centrosomes of this type need not be considered further in this place. It is a gratuitous assumption to hold that the multiple inclusions which are found in many cells in cancer are due to the division of centrosomes, and to explain it we must again draw upon the mystical resources of cancer cells, and to the action of the same unknown toxines that call out degenerations and secretions.

The extremely minute inclusions which have been found in cancer cells and which I have shown in Figures 9 and 10, have been frequently interpreted as minute bits of chromatin or as separated chromosomes. Thus Ruffer and Walker ('93) considered them in this light and agreed with the earlier work of Hauser ('90) in the belief that the so-called Russell's bodies are nothing but portions of the nuclear material which have been derived in one way or another. That there is some good basis for this assumption is indicated by the fact that chromosomes are frequently known to get separated from the nuclei in which they belong and to lead an independent existence in the cell protoplasm. Thus Juel showed that a separated chromosome forms a nucleus of minute size in the cell; it loses its homogeneous appearance in the same way that the chromosomes within the daughter nuclei do, and Juel showed that it forms a reticulate nucleus with chromatin network of the same type as the larger nuclei. Its ultimate fate was not followed. Similar isolated chromosomes have been studied by Boveri and others, and the possibility of such an origin here is not excluded.

Again, the nucleus frequently appears wrinkled and warty and minute protuberances which appear to be on the point of detachment are frequently observed; these are often parts of leucocyte nuclei, the structures par excellence for fragmentation. Figures 57 and 58 show the possibility of such an origin of certain of the minute forms in the cancer cells, and possibly other minute structures, which I can not interpret, may have such an origin (e. g., Figure 37).

Without denying the possibility of such an origin for many of the X-bodies, I do not think that the explanation covers all of them by any means. The origin from a chromosome or from a portion of the resting nucleus might explain the nuclear part of the inclusion, but it fails to indicate the origin of the cytoplasmic part, or the nuclear granulations and the general cell-like structure of the minute inclusions, or of the motile forms as shown in Figures 13 and 30. The inclusions are not like nuclei as described by Juel, but are perfect little cells, each with its minute granular nucleus and alveolar cytoplasm.

Several observers have attributed the X-bodies to secretions in the cell. Pianese, Hansemann, Greenough and Nösske all have a more or less definite conception of the process by which the secreted matter may become hardened or more dense and so give rise to chromatin-like portions. Greenough refers them to specifically modified protoplasm belonging possibly to the cellular structures which Prenant described, and according to whom the protoplasm of a tissue cell contains many kinds of "higher" protoplasm, quite unintelligible to the ordinary cytologist. Nösske interprets the

X-bodies as nothing but vacuoles in the cell which become filled with some kind of inner secretion which is capable of a coagulation process, through which the nucleus-like granules are obtained. In all of these cases there is the same uncertainty of the *modus operandi* in the formation of the questionable body; in each case there appears to be an *a priori* conception of how it might take place, but we search in vain for morphological light on the process. In the meantime the structures are there, definite, clearly differentiated, and of practically the same type, so that they are unmistakable. According to this theory their presence in the nucleus involves that organ of the cell in a new rôle, viz: a "secretion-receptacle," while the unmistakable signs of movement on the part of the inclusions will necessitate a new manifestation of mental agility on the part of the secretionists. My own experience does not enable me to picture secretions in these manifold rôles and in the varied positions, and until more positive evidence is adduced than the literature affords at present, the balance of the evidence must go against this interpretation also.

The X-body thus is not satisfactorily explained as a nuclear product, nor as an astrosphere or secretion. Can it be referred to degeneration products of invading blood cells? The form of the minute inclusion is similar to that of the stage in degeneration where the nucleus is compacted into a homogeneous sphere with cytoplasm in the form of pseudopodia, while the multinucleate condition is duplicated by fragmentation of the nucleus of the degenerated leucocyte. Late stages of the degenerating blood cell may be strikingly like some inclusions which might be mistaken for stages of the X-body. Thus Figure 16 and Figure 17 are very similar, and despite the absence in the latter of the enveloping capsule may be identical in origin. The inclusion shown in Figure 17 is apparently free and resting on two cells, and as it appears under the microscope it is not an intra cytoplasmic structure. This may account for the absence of capsule like that of Figures 16, 23, 38, etc. These are very different things from the more minute and delicate structures shown in Figures 12, 15, 18, 19, 31, 32, 44, etc., and I do not see how the latter can be referred to degenerating blood cells unless, indeed, they are mere fragments of leucocytes; they have no capsules, they are provided with definite chromatin granules and have a well-defined protoplasmic structure. It is an easy way out of a difficulty to say that such things are derived from degenerating tissue elements, and such statements too often given as mere dicta, can not advance the subject in any way.

It is equally unscientific to maintain on the evidence thus far obtained, that these things are parasites. As a working hypothesis, however, it is perfectly legitimate to interpret the various forms of the X-body as organisms quite different from any products of histogenesis. Thus Figures 13 and 30 represent a type of these cell-inclusions which the trained microscopist can not pass over lightly as degeneration products, secretions, or other ordinary cell derivatives. The definite contours, the brightly staining points of chromatin, the evidences of spontaneous movement, all tend to strengthen the hypothesis of a foreign intra-cellular organism. It is conceivable that the structure represented in Figure 13 has been drawn out by division of the cell, and that the connecting strand was not severed after complete separation of the daughter cells, but this seems hardly possible when we consider the age of these cells as shown by the nuclei and cell membranes. The cell plate forming between the daughter cells would certainly have ruptured the delicate connecting strand of the inclusion. The vacuole at the lower end, and the absence of the vacuole at the upper end indicate movement towards the latter.

Notwithstanding the many significant forms assumed by the X-body, I do not yet share the certainty of those who have felt sure enough of the parasite to give it a specific name and a specific systematic position in the animal or vegetable series. The wrecks of *Rhopalocephalus carcinomatosus,* of *Cancriamœba macroglossa,* of Sjöbring's *Strombodes,* indicate the dangers of such attempts, and Feinberg certainly takes great risks in giving to the X-body the specific name of *Histosporidium carcinomatosum.* Even though the X-body were an undoubted organism, which, by the way, is not evidenced in any degree by Feinberg's descriptions, there is absolutely no structural or ontogenetic evidence to justify its position as a sporozoon. There is not enough structural evidence even to place it definitely as plant or as animal and such attempts are premature. If on the evidence thus far advanced we were inclined to indicate relationship of the possible parasite, we would, with Behla, place it with the Chytridiaceæ, or with some other similar group intermediate between the lower plants and protozoa. Even were it proved to be an organism it could be accurately placed only after knowledge of the full life history, and the evidence even for simple reproduction by spore-formation, the possibility of which is barely indicated by structures similar to that represented by Figure 47, is not yet forthcoming. The difficulties of the problem make it the more fascinating, and the parasite hypothesis should not be deprecated or neglected because of the hasty conclusions which some of the advocates of that hypothesis have published. I am much more favorably impressed with the breadth of view of Borrel which prompts him to say: " The criticism which I have just made against the attempts to explain these inclusions as sporozoa should not prevent further research along the lines of this seductive hypothesis " (op. cit. '01, page 60), than with the dogmatism of Marchand when he says: " Since the theoretical considerations are totally opposed to the probability, and indeed the possibility, of a parasitic origin of carcinoma and other malignant neoplasms, and since all cell-inclusions which have been described as parasites are not such but are products of the cell itself, it is high time that pointless research for a carcinoma parasite should be given up and the cause of the malignant growth sought for in other ways."* Even though a specific parasite in carcinoma on *a priori* grounds seems untenable to some, the possibility that such is the case is at least conceivable, and this line of attack should not be neglected until the ætiology of cancer is established. But the *a priori* arguments which have been given are not sufficient to show that this theory is untenable, and the opponents of the theory do not take into consideration all of the possibilities of activity of living foreign inclusions.

Protozoa, like the bacteria, produce substances as products of their metabolic activity which are poisonous to their hosts, and give rise to malignant diseases. Malaria, smallpox, scarlet fever and the several types of disease caused by different species of the genus Trypanosoma owe their morbid effects to toxines of this cellular origin, and these affect the host in various ways, sometimes only physiologically, sometimes both physiologically and morphologically. The specific effect produced in carcinoma is the increased activity of cell division in epithelial cells leading to proliferation of these cells, to abnormal structures and to invasion along the lines of least resistance. The usual response of a lower animal to the intercellular protozoon parasite is the formation of an enveloping sheath or cyst due to accumulation of the connective tissue and called out by stimulus of the parasite. In some cases, however, there is a stimulus to division of the epithelial cells and unusual proliferation in the vicinity of the infection. Thus Thelohan, Hofer and Doflein emphasize the fact that Myxobolus-infection in fish results in the unusual proliferation of the muscle-cells; Leuckart called attention to the epithelial proliferation in Coccidia-infected rabbits, an observation which Tyzzer ('02) has recently confirmed. In vegetable protoplasms the effect of *Plasmodiophora brassicæ* upon plant tissue is the active proliferation of cells and the formation of great deformities in the roots. Proliferation therefore is a general phenomenon attendant on the presence of protozoon parasites.

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*Quoted from abstract in Centralbl. of Bakt. Par. 1903.

The objection has been raised that the supposed parasites in carcinoma are so few in number that they are totally inadequate to account for the active increase of the epithelium, and as a counter-proposition it has been urged, especially by Cohnheim, that the invading epithelium is a reserve of embryonic cells which have lain quiet in the body until the proper conditions bring out the latent energy and cause them to rapidly divide like young cells. I am not competent to discuss this theory, nor to criticize it from the embryological point of view, and for this reason the fact that it seems to me farfetched and improbable, will carry little weight. I am more at home in criticizing the recent view of Ribbert ('01), who makes the objection that if parasites are the cause of cancer they must have the very novel power of changing the entire nature of epithelial cells; that is, of generalizing them and of making them more perfect cells instead of more disabled cells, or that the parasites instead of exerting a harmful effect upon the cells they infest produce a beneficial effect. A satisfactory response to this kind of argument was made by R. Hertwig (1900), who called attention to the fact that cancer cells instead of being perfect or embryonic in nature, are actually the reverse and show unmistakable signs of senile degeneration. He called attention to the fact that in protozoa (Actinosphærium) after a long period of rapid asexual reproduction the cells become hypertrophied and abnormal in structure; the nuclei are much enlarged and show characteristic morphological changes which are due to protoplasmic senility. The fact that such changes are frequent in cultures of protozoa which have been kept from sexual reproduction for long periods has been known for many years, and old age in these single cells is a well-known phenomenon since Maupas' classical researches in 1888 and 1889. Hertwig's contention is that abnormal epithelial growths do not occur in the young human tissues, but make their appearance after cell-proliferation has become less active. In such cancer cells, the abnormal growth is a sign, not of youth with its well-balanced self-regulation which prohibits abnormalities, but of senility in which the self-regulating power so characteristic of all living things has been lost. Secondary evidences of this degeneration are shown by the hypertrophied nucleus, frequent amitoses and loss of the specific characteristics of the tissue involved. The problem of the ætiology of cancer deals with the direct cause of this special increase of the dividing energy of cancer cells.

An epithelial cell in an adult human being represents the Nth generation of cells since the first cleavage of the egg, and just as we are justified in saying that the 319th generation of Stylonychia pustulata, or the 200th generation of Paramœcium caudatum represent the limits of dividing energy of these two organisms, so we may say that the Nth generation of any tissue cells represents the limit of dividing energy in these human cells. In the case of Paramœcium, however, it has been shown that the waning energy may be renewed by artificial means, and that the addition of a little salt (potassium phosphate) to the medium in which they live for the short period of thirty minutes, was sufficient to stimulate the worn-out cells to a renewed activity, and instead of dying like the organisms not thus treated they divided about 200 times more. This process was repeated again and again until the race finally succumbed in the 742d generation (see Calkins '04). The dividing power of the worn-out cells of an epithelium is capable of similar reinvigoration, even after the limits of growth have been attained, and renewed proliferation may take place in functional epithelium, giving rise to abnormal growths which have been explained without calling upon improbable and inexplicable embryonic tissues.

The stimulus to the renewal of the dividing activity of an epithelial cell in the case of carcinoma may very well come from the inclusions, notwithstanding the relatively small numbers present. Owing to the small size of these inclusions I am inclined to think that they are frequently overlooked, and that their scarcity is somewhat traditional. I have found them in abundance in a rapidly growing carcinoma where they are supposed to be less numerous than in the older, more slowly-growing types. If the analogy with the worn-out race of Paramœcium holds good for the epithelial cells, then would it be unnecessary to have a parasite for each cell as Ribbert assumes; a single stimulated cell, if reacting like Paramœcium, would divide again and again after stimulation, and the effects of the parasite would be a Fernwirkung in a new sense.

In a certain sense the stimulus which a parasite might thus produce is not unlike the action of a spermatozoon in the egg. In relative volume to the egg cell the male cell is no greater than the relative volume of the X-body to the epithelial cell, and yet it produces the wonderful result of development. Its action has been interpreted as an effect of enzymes, and in support of this explanation the work of Loeb and others on artificial parthenogenesis has been cited. The same effect as that of a spermatozoon has been produced by a minute quantity of magnesium chloride, and the stimulus has thus been given for the egg to divide again and again, while self-regulation and differentiation work together to mold the cell progeny into the form and structure of the organism. Here then is the temporary action of a foreign substance upon living protoplasm, the effects of which are felt long after the direct contact, and another instance of this special type of Fernwirkung.

If a toxine of this type is at work in carcinoma it would not be unreasonable to assume that a certain optimum is necessary to produce the stimulating effect; if this optimum is exceeded, growth may be retarded; if the proper amount is not present the effects will be nil. In the experiments with Paramœcium I found that a certain quantity of salt and a certain time of its action were necessary for successful results, and the same experience has been met with by all who have attempted to induce artificial parthenogenesis in eggs. This may be one explanation of the slow growth of cancers in which the number of the X-bodies in the epithelial cells is relatively large. Upon such an hypothesis it may be seen that while possible carcinoma parasites have no such effect as the smallpox organisms for example, such parasites might be quite sufficient to stimulate cell development and tumor formation while the ensuing morbidity and ultimate cachexia might well be traced to general poisoning due to a disordered system and not directly referable to the parasites themselves.

SUMMARY.

1. There are two main groups of inclusions in carcinoma, those which are encapsuled, and those without capsules.

2. Encapsuled inclusions appear under several forms, sometimes definitely cellular in which case they are recognizable as blood cells or epithelial cells, sometimes variously modified by degenerative changes and unrecognizable.

3. The various modifications of such inclusions have been variously and erroneously interpreted as stages in the life history of protozoan parasites.

4. Attention is directed to the fact that parasites in cell-protoplasm can not get food nor rid themselves of waste products when enclosed in capsules.

5. The same principle may account for the degeneration of encapsuled invading cells such as leucocytes, lymphocytes, etc.

6. Capsules or cysts around parasites are formed not by the host cells but by the parasites at periods of reproduction or for protection

of spores, etc. Capsules around cell inclusions in carcinoma are formed by the protoplasm of the invaded cells.

7. Non-encapsuled inclusions in the form of secretions, degenerations, metaplasmic modifications, etc., are likewise present in cancer cells. These must be correctly interpreted before any parasite hypothesis is advanced.

8. The X-body of Behla is not satisfactorily explained as metaplasmic, degeneration or secretion product, although it is not yet known to be independent from blood-cell origin. It appears without a capsule but lies in a vacuole in the cytoplasm or nucleus. It possesses a granule or granules which take up the chromatin dyes with marked intensity. In some cases it shows strong evidence of spontaneous movement.

9. Theoretical considerations are not entirely sufficient to outweigh the morphological evidences of an organism. The small number of inclusions indicates that no such results are to be expected as those which follow the presence of masses of parasites as in smallpox or scarlet fever, or malaria, but experimental results show that great proliferating stimuli may be given by minute quantities of poison, and *a priori,* grounds are thus obtained to justify the effect, in this case a tumor, through the activity of a relatively few parasites.

10. The proliferating epithelial cells of carcinoma do not show the characteristic of embryonic cells, but rather of senile degenerate cells as shown by cell and nuclear hypertrophy, and by loss of the self-regulating power which is particularly characteristic of young or embryonic cells.

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DESCRIPTION OF PLATES.

All figures are drawn with the camera with lens system of ocular No. 6 Zeiss and Objective 1.5 Zeiss. Magnification about 1,800 diam. All inclusions represented are from one case of carcinoma.

Plate I.

Fig. 1. A cell-inclusion of unknown origin but probably a degenerating epithelial cell.

Fig. 2. Epithelial cell with invaded blood cell (lymphocyte).

Fig. 3. Cytoplasmic degeneration beginning in invading leucocyte. The nucleus of the invader is not yet affected.

Fig. 4. Degenerating lymph cell in which no trace of cytoplasm remains.

Fig. 5. Further stage in the degeneration of blood cell. The chromatin of the nucleus is massed, although not so densely as in the preceding figure.

Fig. 6. Late stage of nuclear metamorphosis of an invading blood cell. The thickness of the capsular rim is characteristic of the inclusions in this stage.

Fig. 7. Colloidal masses in a vacuole with capsule, representing possibly, the end phase of degeneration of an invading cell.

Fig. 8. A "Plimmer's body" consisting of vacuole, central spot and rim. Indicating no differentiated granules like those of the X-body.

Fig. 9. Homogeneous spot of foreign substance in cytoplasm, characteristic of many inclusions in the epithelial cells.

Fig. 10. Similar body somewhat larger, with a characteristic vacuole.

Fig. 11. Two "Plimmer's bodies" with central, differentiated spot.

Fig. 12. The X-body of Behla, indenting the nuclear membrane, and with highly characteristic form including pseudopodial processes which may be traced to the periphery of the vacuole, and an internal differently staining spot which is always sharp and clearly defined.

Fig. 13. A form of the X-body which was seen only two or three times. It stretches between two adjacent cells and each enlarged end contains three distinct spots which take the chromatin dyes.

Figs. 14 and 15. Two characteristic X-bodies.

Figs. 16 and 17. Two similar amœboid masses with definite granular masses of chromatin-like substance. One has a capsule, the other has none. These cannot be organisms but their origin is not made out.

Fig. 18. An X-body partly embedded in the polymorphic nucleus of an epithelial cell.

Plate II.

Fig. 19. Epithelial cell with leucocyte invader, and minute X-body of characteristic form and content.

Figs. 20 and 21. Two stages in the degeneration of an inclosed blood cell. In the latter the nucleus is becoming homogeneous and contracted through the loss of nuclear fluids.

Figs. 22 and 23. Two later stages in the degeneration of invading blood cells. The compact nucleus is much reduced in size and the cytoplasm appears broken.

Fig. 24. Last remains of what is apparently the end stage in degeneration of the inclosed cell; nothing is present but a homogeneous central dot, while the remainder of the cavity inside the capsule is flocculent and unformed.

Fig. 25. A figure representing a late stage in the degeneration of a cell with chromosomes. These are the "tadpole" forms described by Sawtschenko and interpreted as parasites in the slime formed by a degenerating cell.

Fig. 26. A cell with nucleus broken into many fragments which appear uniform in size and characteristic enough to be regarded by many as stages in the reproduction of a parasite.

Fig. 27. Fat-like particles in an epithelial cell showing fusion of droplets.

Fig. 28. Nucleus of epithelial cell with several questionable bodies in the cytoplasm and one similar body in the nucleus. Suggested as a possible early phase of the intra-nuclear X-body.

Figs. 29-33. Various phases of the X-body. In the last two the chromatin material is in the form of minute granules resembling the nuclear conditions of many lower forms. In figure 30 the X-body has the form of an amœboid organism apparently in the process of migrating from the vacuole into an adjacent epithelial cell. It is very difficult to interpret this form as a degeneration product or portion of a leucocyte. The chromatin material is in four separated granules, one at each end of a pseudopodia-like process.

Fig. 34. A stage in the anaphase of mitosis showing chromosomes and spindle fibres but with no trace of the X-body at the poles as would be expected were Borrel's centrosome-hypothesis correct.

Fig. 35. An intra-nuclear X-body with typical structure.

Fig. 36. An enlarged epithelial cell with hypertrophied nucleus and curious neck-like process; a type of cell which was supposed by Eisen to be a specific parasite of cancer and named by him "Cancriamœba macroglossa."

Fig. 37. A cell with a minute inclusion of unknown origin. The appearance of the minute elements within the vacuole suggests that it is derived from a misplaced chromosome, but not enough stages were observed to warrant this interpretation.

Plate III.

Fig. 38. A stage which is difficult to interpret without comparison with the earlier and similar figures. It represents a rather late stage in the degeneration of an invading cell with the compact nucleus undergoing secondary vacuolization preparatory to the formation of numerous granules as represented in figure 6.

Figs. 39-42. Stages in the metamorphosis of the "nucleolus" of epithelial cells, showing the hollow sphere, the reticulated nucleolar body (figure 40, compare with this figures 53 and 54) the clathrate body and the degenerate vesicular body with change in color reaction.

Fig. 43. A stage analogous to that of figure 24.

Figs. 44, 45, 46. Various stages to show the nature of the chromatin in the X-body in the form of separated granules, sometimes of small size, sometimes large.

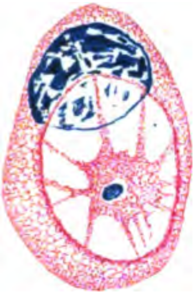
Fig. 47. An interesting stage suggestive of reproductive process in some single-celled organisms. Each chromatin granule has its separate bit of cytoplasm.

Figs. 48-52. Similar types frequently found in this cancer.

Figs. 53-56. Characteristic intra-nuclear forms which are identical in structure to the X-bodies in the cytoplasm. Possibly to be identified as degenerations of the nucleolus as shown in figure 40. Figure 56 is a curious structure which were it not isolated, might suggest a stage in merozoite-formation of a coccidium, but is probably only a particular type of nuclear degeneration.

Figs. 57 and 58. Two nuclei with buds showing the possible mode of origin of structures like those shown in figure 10, etc.

Fig. 59. Another mitotic figure in the metaphase stage showing that the spindle-poles, although provided with centrosomes have no trace of astrospheres.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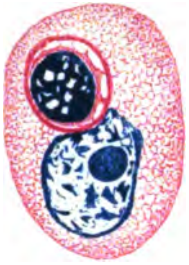
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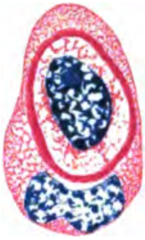
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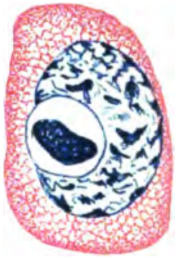
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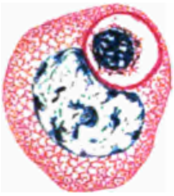
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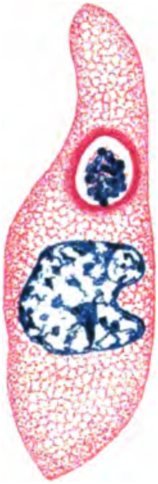
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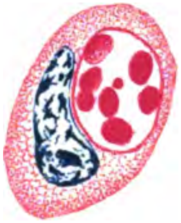
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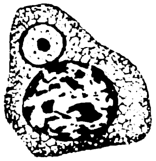
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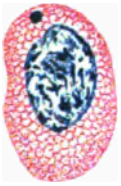
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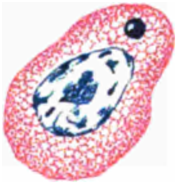
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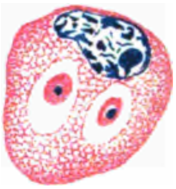
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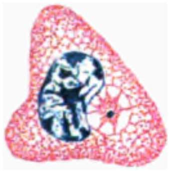
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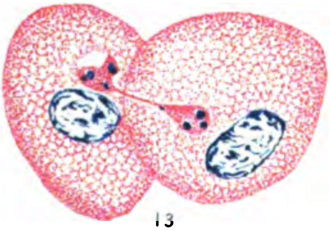
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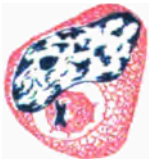
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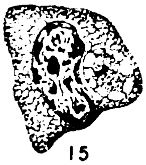
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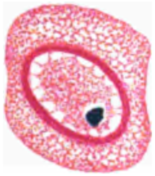
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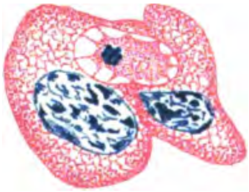
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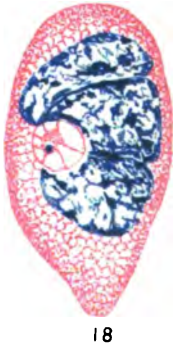
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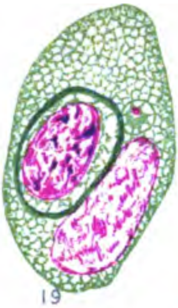

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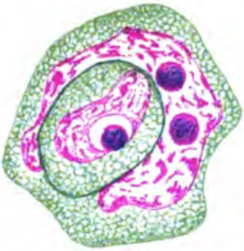

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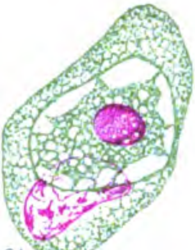

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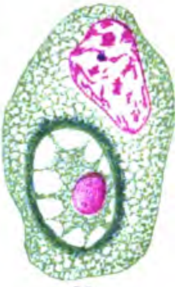

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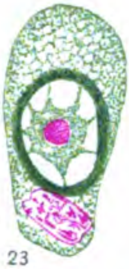

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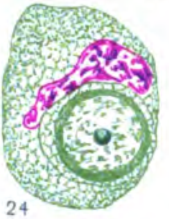

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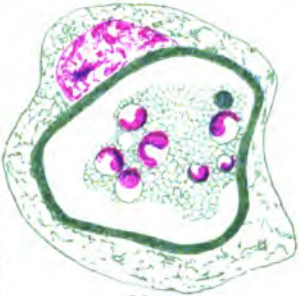

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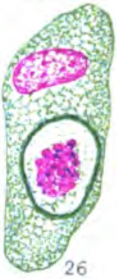

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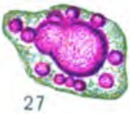

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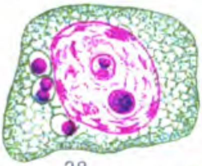

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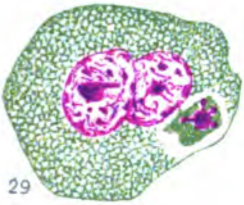

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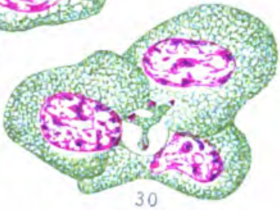

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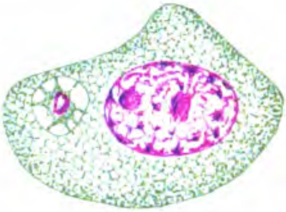

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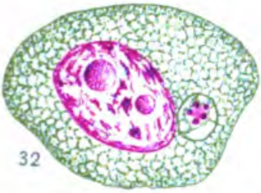

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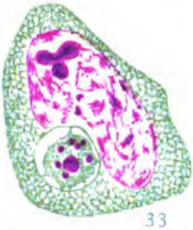

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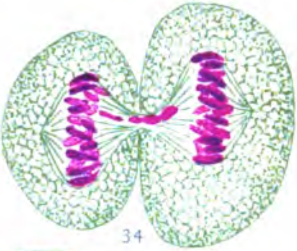

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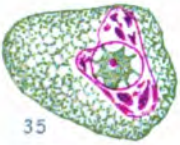

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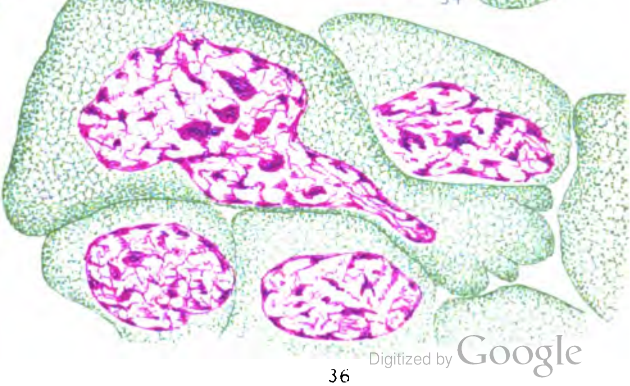

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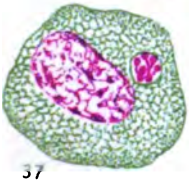

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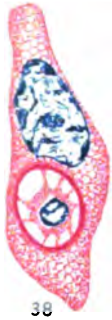
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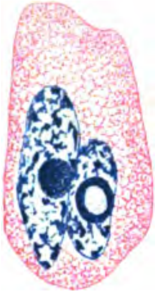
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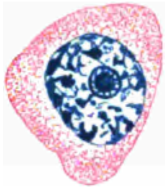
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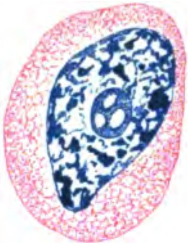
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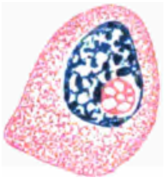
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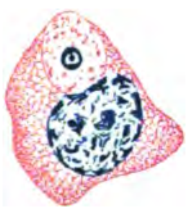
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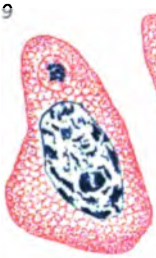
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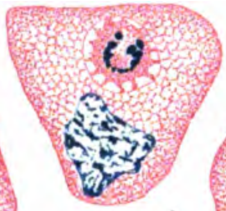
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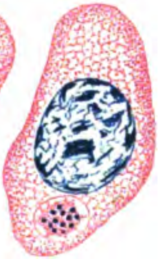
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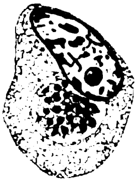
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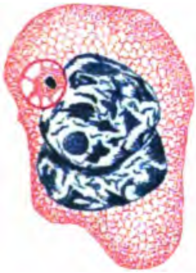
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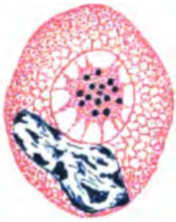
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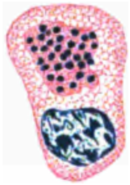
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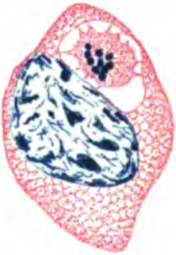
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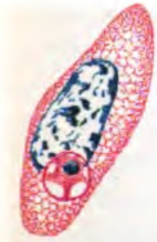
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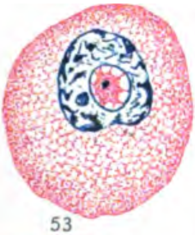
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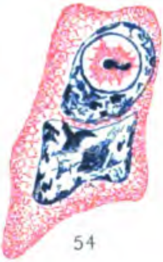
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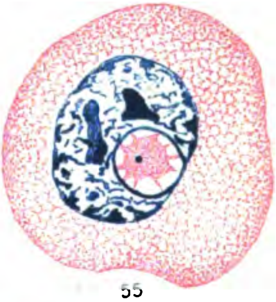
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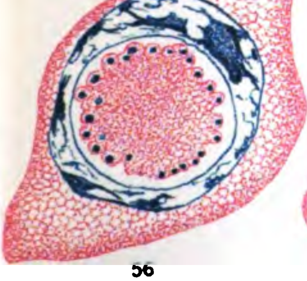
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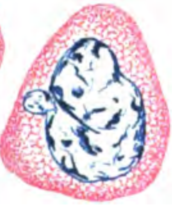
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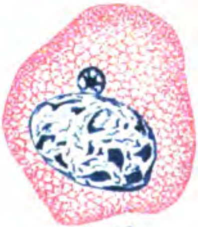
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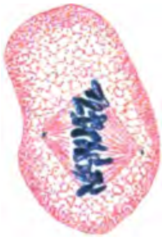
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ON THE ANALOGY BETWEEN SMALLPOX AND CANCER.

By H. R. Gaylord, M. D.

The idea that certain points of similarity exist between the cancerous process and the acute exanthemata, especially smallpox, is by no means new. Attention has been called to the subject from two entirely different points of view, both, however, having as their basis the infectious nature of cancer. Those who have been engaged in the study of the cell-inclusions of cancer and vaccine and find in these inclusions certain points of resemblance were the first to call attention to the analogy existing between the two problems. The second point of view is as yet represented by Borrel, who has recently attempted to show that without regard to the question of the inclusions (which he views as often characteristic of the process but not as parasites), certain points of similarity can be traced which lead him to the conclusion that although the nature of the agent is as yet undiscovered, both the epithelioses and epithelioma are infectious and may be said to possess a virus which is capable of transmitting the disease. He supposes the existence of a cancer virus, which is suggested to him by results recently obtained in the transplantation of epitheliomata in mice. Regarding the similarity of the virus found in smallpox and the virus obtained in sheep-pox (clavelée), Borrel brings many points of interest, especially certain experiments with the latter which is found to pass through coarser grades of the Chamberlain filter but not through the finest.

Without entering here into the details of Borrel's research, to which we shall later have to refer, we may say that he favors the idea that in both cancer and smallpox the infective agent is a minute, if not invisible parasite, and that the inclusions are the result of degenerative changes in the protoplasm or of invaginated

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leucocytes in process of disintegration. He finds in sheep-pox a characteristic inclusion in the cytoplasm of the epithelial cells, and in an epithelioma of the mouse which he used for transplantation, similar inclusions. The inclusions found in the corneal epithelium of the rabbit after inoculation with vaccine, he believes with Salkowski, can be reproduced by irritants such as diphtheria toxin, or superheated vaccine.

In the light of the recent work of Councilman, Brinckerhoff and Macgrath, and the elucidation and confirmation which their work on vaccine and smallpox has found at the hands of Calkins, it would be profitable to recall the points of resemblance which may be traced between cancer and these processes on the basis of the inclusions found in both.

The first author who especially called attention to the similarity of appearance between certain forms of the so-called vaccine body or Cytoryctes vaccinæ (Guarnieri) was Gorini.* This author pointed out that in inoculated corneæ certain larger forms of the vaccine body were frequently surrounded by a form of capsule, and that they then closely resembled the cell-inclusions of cancer. The forms in question in vaccine had been described previously by L. Pfeiffer, Guarnieri and Clarke and were interpreted by these authors as a more advanced stage of development of the smaller and characteristic vaccine body. A gradual transition between the typical vaccine body and these larger inclusions could be traced by Gorini, who concluded that they are developed from the smaller inclusions. These larger bodies he finds possess a high degree of similarity in form and appearance to the cell inclusions of cancer.

In 1900 we were led to conclude that the inclusions of cancer and those of vaccine were essential in their nature, although not identical, and reinforced by the publication of Wasielewski† who, to our mind

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*Gorini. Centralbl. f. Bakteriologie Abt. I, Vol. 28, 1900.

† The conclusions of Wasielewski are so important that it is not out of place here to give them in full:

Contribution to Our Knowledge of the Nature of Vaccine.—Von Wasielewski Zeitschrift für Hygiene, Vol. XXXVIII, Part 2, October, 1901.

"*Conclusions.* The vaccine bodies are the single characteristic structures which are found in the epithelial cells of the skin and mucous membrane brought all but convincing proof of the parasitic nature of the Cytoryctes vaccinæ, we arrived at the conclusion that the conditions surrounding both problems were identical and that if the inclusions in vaccine were parasites, the inclusions in cancer were likewise parasites.

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in vaccine and variola. These are absent in normal epithelial cells of the skin and under all other conditions. The bacteria described as the specific factors in vaccine are saprophytes and have no etiological significance as shown by the efficiency of lymph entirely free from bacteria.

"2. The characteristic vaccine bodies invariably appear in the corneal epithelium of rabbits inoculated with potent vaccine.

"3. The same structures can not be induced in the epithelial cells of the cornea in rabbits in any other way.

"4. It is impossible that the vaccine bodies are leucocytes or degeneration products of leucocytes.

"5. The derivation of these bodies from the nuclei of the epithelial cells is disproven by, first, their appearance in perfectly normal cells; second, their presence in cells which are undergoing mitosis; third, by the appearance of the smallest of these bodies at the periphery of the lesion, where they make their first appearance in the peripheries of the cells most widely distant from the nuclei.

"6. The origin of these bodies from the cell protoplasm as the result of the specific action of an organism supposedly invisible because of its minuteness, can not be proven or entirely disproven but is rendered throughout improbable on the following grounds: *a.* Because the specific factor of vaccine can be removed by filtration for which reason there is no ground for supposing it to be an infinitely minute organism. *b.* Because the vaccine bodies appear in cells undergoing division in which the karyokinetic figures show the characteristic protoplasmic radiation and the figures themselves are derived from the central portion of the cytoplasm, which according to the views of Hueckel is first affected by the specific action of vaccine. *c.* Because a similar specific transformation involving alone special portions of the protoplasm has never been proven in any other case. *d.* Because the extensive and rapid appearance of degeneration and vacuole formation as that of the granulated degeneration of the vaccine bodies has never been observed in any of the degeneration processes of the protoplasm, because the development of such characteristic cell-inclusions can not be compared with any known form of cellular degeneration. *e.* Because the impotent filtrate of vaccine lymph has no specific toxin action upon the epithelium.

"7. Size, form and structure, distribution and extension at the point of inoculation, as well as the appearance of evidences of division and degenera-

Our analogy extended likewise to the examination of fresh material in which we found certain hyaline bodies which we compared to the so-called fresh vaccine body.

On the same date of the same year Bosc* published an article advancing exactly the same idea but including sheep-pox, a disease which we had not been able to study. Bosc went so far as to classify these diseases and believes that they form a group of sporozoan infections. Bosc gives an exhaustive description of sheep-pox, the virus of which causes epithelial lesions and connective tissue nodules in the subcutaneous tissues. In the exudate obtained from fresh postules he found characteristic epithelial cells containing highly refractive bodies embedded in the protoplasm and similar free bodies, many very small, which presented characteristics similar to the inclusions in the cells. He finds that some of the inclusions and free bodies take the nuclear stain, although irregularly. In

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tion forms, speak for the supposition of Guarnieri that the vaccine bodies are parasites (schmarotzi) within the cells.

"8. The changes which the presence of the vaccine bodies induce in the cells support this view.

"9. The propagation of vaccine through forty-eight generations by inoculation on the cornea of rabbits proves that an active continuous proliferation of the vaccine bodies occurs at the site of inoculation.

"10. That, besides the vaccine bodies at the point of inoculation, no other organisms are either microscopically or bacteriologically demonstrable, and the constant reappearance of the vaccine bodies to the forty-eighth generation is demonstrable."

The author concludes that the supposition of Guarnieri that the vaccine body is the vaccine organism must be viewed as extremely probable.

The importance which we attached to Wasielewski's work and our point of view is shown by the following paragraph taken from the manuscript of an address given by us in Detroit February 21, 1902, before the Wayne County Medical Society: "It is manifestly impossible to apply all of these conclusions to the organisms of cancer, inasmuch as the experimental basis in cancer is infinitely more limited, but by a realization of the intrinsic nature of the two problems, it may readily be seen that the definite establishment of the vaccine body as the vaccine organism places us a great step forward in an appreciation of the true significance of these characteristic cell-inclusions in cancer."

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*Bosc. Archives de Medecine Experimentale. Vol. XIII, No.3. May, 1901.

many, however, the central portion of the body takes a chromatic stain. Similar inclusions are to be found in the cells forming the connective tissue nodules. *The inclusions vary in size from bodies not larger than a micrococcus to large structures either spherical or of irregular form with a more refractive central body.* All the inclusions are surrounded by a more or less clearly defined zone of clear protoplasm. Bosc inoculated the cornea in a number of sheep and obtained a lesion similar to that of vaccine, the inclusions being very abundant by the sixth day. As controls he inoculated the cornea with pathogenic bacteria, fungi, dust and *superheated sheep virus.* In no case were inclusions formed which could be compared to the inclusions following inoculation with the fresh virus.

Without going further into the details of Bosc's paper, to which the reader is referred, it may be seen that he definitely establishes the presence of characteristic inclusions and shows that in this connection the virus is similar to that of vaccine. Neither Wasielewski nor Borrel describes intranuclear inclusions in vaccine or smallpox, but Bosc* has since published a paper on the parasite of variola which appeared the 24th of October, 1903, describing practically all the forms observed by Councilman. His paper, although appearing several months after Councilman's preliminary announcement, affords an independent observation of these structures. Thus far in sheep-pox no intranuclear forms have been observed.

The only reference to nuclear inclusions in vaccine, which in the light of Councilman's work receives a renewed interest, is that of Gorini.† Gorini found that certain forms of the Cytoryctes vaccinæ could be found within the nuclei of the epithelial cells of the inoculated cornea, and that many of the inclusions were so placed in relation to the nucleus that they must be looked upon as either penetrating or being cast off from the nucleus. Many of the intranuclear inclusions are of the larger variety; i. e., those already referred to as closely resembling the inclusions of cancer. Gorini concluded that either the Cytoryctes is a parasite which infects the nucleus and in many cases passes then to the protoplasm (or the reverse), or that the Cytoryctes is not a parasite but is a characteristic body derived from the nucleus. Which of these hypotheses he favors Gorini does not state.

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*Bosc. Comptes rendus des Séances de la Société de Biologie T. LV. p. 1204.

†Gorini. Centralblatt f. Bakteriologie. Abt. I. Vol. 29, 1901.

The presence of typical birdseye inclusions within the nuclei in cancer was first noted by Steinhaus.* A simple reference to his illustrations clearly defines this point. Plimmer† likewise found the same inclusions in nuclei and gave an illustration representing this phase. Their presence has also been noted, described and illustrated by Apolant and Embden, who in contradistinction to the above-mentioned authors, who held them to be parasites, believed that many of the birdseye inclusions are derived from the nucleus as the result of degenerative changes. In fact, Apolant and Embden believe that the birdseye inclusions in the protoplasm and the birdseye inclusions in the nuclei are produced by different processes and can not argee with Nösske that the only source of the inclusions is the degenerated protoplasm. This criticism applies to Greenough, who preceded Nösske in attributing these inclusions to secretive processes in the protoplasm. The presence of inclusions in the nucleus likewise diminishes the value of such experiments as those made under Aschoff's direction by Spirlas, in which the introduction of foreign cells into the peritoneum of animals leads to the formation of inclusions in their leucocytes which present a similarity to birdseye inclusions. These experiments tend to show that the inclusions are the result of foreign bodies entering the cells from without. They must also be found in the nuclei of the leucocytes, if Aschoff's criticism is to hold. For the nucleus these authors assume that the birdseye inclusions are formed in various ways. They assume these bodies are formed within the nucleus and that the central body which is often acidophile is derived from the nucleolus of the cell, which is basophile, by some process which simply changes its staining affinity. The inclusions with acidophile central bodies are in appearance identical with the inclusions in the protoplasm. They suggested that in many cases extrusion of the nucleolus with transformation of its staining affinities might account for the formation of some of the inclusions in the protoplasm. The idea that the inclusions with acidophile central bodies might have penetrated into the nuclei from the protoplasm, in which case it is not necessary to assume a mysterious transformation of the staining affinities of the central body, does not of course occur to these authors, whose purpose is to show that the inclusions are derived from the constituents of the cells and have not entered from without.

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*Steinhaus. Virchow's Archiv. Bd. 126.

†Plimmer. The Practitioner. April, 1899.

Borrel, who appears for the present to lay less stress on the centrosome as the origin of the inclusions, believes on the other hand that in most cases they are metamorphosed leucocytes. That it is manifestly impossible to prove that an object in a hardened section is passing in either direction must be apparent. It would seem, however, that unless we assume a multiplicity of sources for these so characteristic inclusions we are not able to satisfy the many observers who have studied them from other than the parasitic standpoint. On the other hand, those who view them as parasites offer but one explanation, and are reinforced by the presence in other recognizedly infectious processes of the epithelium of inclusions likewise viewed as parasites, which they closely resemble.

In our original publication* we noted the presence of intranuclear inclusions but devoted our attention more definitely to the cytoplasmic forms. Since the publication of Councilman's article Calkins has demonstrated the frequent occurrence of typical intranuclear birdseye inclusions, thus confirming the observations of Steinhaus, Plimmer and Apolant and Embden. The work of Councilman, showing the presence of a complete cycle of an intranuclear organism in smallpox, must undoubtedly call attention to the importance and shed light on the probable significance of characteristic intranuclear inclusions in cancer. If the restrictions of Apolant and Embden are correct in the case of the intranuclear inclusions of cancer, they apply with equal force to the intranuclear inclusions of Councilman in smallpox, and Gorini in vaccine. The only essential difference in the two cases, as we have previously pointed out, is that vaccine and variola are accepted as certainly infectious and cancer is not.

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*Third Annual Report of this Laboratory for the year 1900.

The grounds on which most pathologists refuse to see in cancer an infectious process is that as yet all experiments calculated to show the infectious nature of cancer have as their basis the transplantability of the cancer cell. In the case of Borrel, however, the transplantation results appear to have brought conviction of the infective nature of the process. His belief that the inclusions both in cancer and smallpox are not parasites leads him to the hypothesis that the organism of cancer must be of ultra-microscopic dimensions.

For this reason it is well to consider what is the evidence of ultra-minute organisms for vaccine, variola and sheep-pox. The virus of sheep-pox has been shown by Borrel to pass through the Berkefeld and the coarser grades of the Chamberlain filter. Vaccine is supposed not to pass through any of the bacteria-proof filters. We are, however, informed by Dr. Georges Dryer that he has recently succeeded in passing vaccine through Berkefeld bougies which were proof against the bacillus of chicken cholera, recognizedly one of the more minute bacilli. This being the case, vaccine is placed on the same footing as the virus of sheep-pox. Mouth-and-claw disease, which is mentioned by Borrel as one of the filterable infective agents, has, however, nothing otherwise in common with vaccine and has been shown by Wasielewski never to produce inclusions or lesions in the cornea which can in any way be compared to vaccine and sheep-pox. *Should we conclude that in these diseases we are dealing with a micro parasite?*

Borrel in his publication brings light on this point. In his filtration experiments with sheep-pox he discovered that when he diluted the virus with tap water, after four days there developed in the filtered and otherwise sterile virus a small protozoon, to which he gave the name Micromonas Mesnili. The organism when in its largest form in the virus (it is of course impossible to affirm that the organism under other conditions does not possess a still larger phase), was three or four microns long and as many wide. That the organism in question had nothing to do with the virus was shown when distilled or sterilized water was used to dilute the virus. Borrel found that this organism followed the same law as the active principle of the virus, that is, it passed through the filters through which the virus passed and was held back by filters which were proof against the virus. That the organism passed through in some practically invisible spore form and then developed on the suitable medium of the virus was shown by its appearance in virus *only after four days* and the impossibility of detecting it in filtered water in which the larger forms did not develop. Borrel was forced to conclude that " Le passage à travers un filtre n'implique pas forcément l'idée d'un microbe invisible."* With these facts before him, however, Borrel pronounces against the inclusions as parasites and believes that the organism will prove to be a small if not an invisible organism.

It must be noted that Borrel's Micromonas shows as its largest form an organism considerably smaller than the larger inclusions of vaccine, variola and cancer. However, to assume that because the spore of an organism is sufficiently small to pass through a certain filter, its largest form would be within a certain limit of size, is not justified by biological knowledge. Calkins has described a protozoon, Lymphosporidium truttæ, the spores of which are one and one-half microns and divide into six sporozoites each less than one-half a micron in diameter. Borrel likewise points out that there is an essential difference between the smaller forms of motile, animate parasites and bacteria of relatively the same dimensions. The first are more plastic and accommodate themselves to the pores of the filter and pass through where bacteria are held back. In all probability the sporozoites of Lymphosporidium truttæ, less than one-half a micron in diameter, would pass through a bacteria-proof filter and yet the largest form of this organism is a multinuclear amœba twenty-five microns in diameter.

For this reason there is no ground for supposing that the filtration experiments are opposed to the protozoan theory of smallpox. What must we conclude then in the light of Councilman's and Calkin's observations? If these observations are correct we have an intranuclear and protoplasmic parasite of considerable dimensions, which, in one of its phases, passes through a Berkefeld bougie. The cytoplasmic and certain intranuclear forms described by Gorini have a recognized similarity of appearance to the cytoplasmic and intranuclear inclusions in cancer. The difficulty in carrying the analogy further is found in our inability thus far to separate from the epithelial cells in cancer an infective agent capable of producing cancer even in animals of the same species. All of the experiments in cancer have as their basis the epithelial cell, and it has thus far been impossible to apply with success filtration experiments similar to those of Borrel and others in smallpox. For this reason it is difficult to understand why Borrel, who believes in the infective nature of cancer, should seek its solution in an ultra-microscopic organism. It would appear that the closest analogy which we can draw between smallpox and cancer is the presence of the inclusions in the epithelial cells, and it would seem that filtration, even in the light of Borrel's experiments, in no way shakes the conclusions of Councilman and Calkins.

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* Without attempting to discuss all the filterable viruses it may be noted that the blood of yellow fever patients passes through the Berkefeld bougie but not the fine Chamberlain. Without regard to the work of the Marine Hospital Commission which has been so sharply criticised by Carrol, it must be noted that the relation of the mosquito to this disease strongly suggests a protozoon, perhaps an extremely small one.

We have maintained from the first that the solution of the vaccine-variola problem, with the establishment of the vaccine body as the vaccine organism, would bring, as a natural consequence, the establishment of the probable protozoan nature of the inclusions of cancer. The difficulty of the problem in cancer is unfortunately greater than in smallpox, owing to the limitation of experimental possibilities, but the advances which have been made in the study of the inclusions of the exanthemata have already thrown a suggestive light on the question of the inclusions in cancer. As the whole question stands, it must be conceded that the importance of questionable inclusions in processes with obscure etiology has greatly increased, and that the interpretation of the inclusions of cancer as parasites has gained rather than lost ground as the result of the researches of the last two years.

REPORT OF THE BIOLOGICAL-CHEMICAL DEPARTMENT

By G. H. A. Clowes, Ph.D.

A brief summary of the work carried out in the biological-chemical department since the completion of the new laboratory was presented in the Fourth Annual Report. In the absence of sufficient reliable analytical data it was decided to postpone publication of results, especially those bearing upon the metabolism of patients suffering from cancer, until a sufficient amount of confirmatory evidence had been accumulated. The work of the chemical department, either in progress or contemplated, was considered under three main heads: (1) Studies in metabolism regarding the equilibrium of nitrogenous bodies, chlorides, etc., in the system, and including work on the stomach contents, blood and urine; (2) Micro-chemical investigations carried out with a view to obtaining evidence regarding the cause of cancer and the nature of the disturbances taking place in the metabolism of the individual cancer-cells; (3) Attempts to cure cases suffering from cancer by means of serum-therapeutic treatment or chemical agencies, the possible value of which had been indicated by investigations carried out under the two first heads. To these three main lines of work must be added a fourth, to which a considerable amount of attention has been paid in the course of the last two years; the examination of cancerous material and the serum of cancer cases for hæmolysins, precipitines, etc., applying the principles evolved in immunity studies carried out in other directions.

The work included under the heading of metabolism is practically completed and the results obtained are to be found in a series of papers published in this report dealing with the nitrogen and chlorine equilibrium established between the food and excreta of cancer cases, and the influence of varied proportions of salts in the diet upon the blood and urine. In this portion of the work special attention has been paid to the question of improving the nutrition of patients suffering from tumors which from their nature and location are capable of interfering with the proper assimilation of food.

A paper has also been included dealing with the secretions of the stomach in cancer as compared with normal and other pathological conditions and indicating to what extent chemical analysis of the contents of that organ can be utilized for diagnostic purposes, and also endeavoring to throw light on the nature of the disturbances in osmotic equilibrium which lead to an interference with the normal secretions.

In a paper dealing with the possibility of diagnosing cancer by considering the nature and proportion of the various urinary constituents it is pointed out that any indications obtained by this means regarding the location of a tumor are purely the result of secondary interferences with the metabolic equilibrium of the cells of the organs involved, and that no specific cancer reaction can be said to have been obtained by this means.

Abstracts of papers published in other journals dealing more particularly with the physico-chemical problems involved in the osmotic equilibrium of a variety of pathological cases have been included.

Throughout the work referred to above an effort has been made to present a thorough summary of the literature to date of publication, and to point out wherein the work carried out in this Laboratory is in agreement with or deviates from that of previous investigators.

Under the second head of micro-chemical research a paper dealing with the staining reaction of mast cells, etc., has already been published, and further work of this nature dealing more especially with the cancer-cells themselves is already in course of completion. Special effort is being devoted to the problem of obtaining an immunity against cancer, and horses are being employed for the preparation of sera. The results obtained in this direction are not, however, sufficiently positive to permit of any immediate expression of opinion regarding the ultimate probability of success. The possession of a series of cancer mice, kindly put at the disposal of this Laboratory by Professor Jensen, has made it possible to commence experiments on immunity on a much larger scale and with much greater likelihood of success than was possible when dealing with human cases exclusively.

In view of the interest attaching to the employment of X-rays in the treatment of cancer, a short note on the influence which they exert on the metabolism of normal animals has been incorporated in this report.

A series of analyses of mouse tumors taken at various stages of development is being carried out in this Laboratory at the present time. Such tumors, having an absolutely common source, afford a much more satisfactory basis of comparison than can be hoped for from a series of human tumors.

Finally, with a view to possible early diagnosis, tumors and the blood sera of cancer cases have been examined for hæmolysins, precipitines, etc. Whilst certain positive results have been obtained, it has not at present been possible to prove conclusively that the reactions in question were directly dependent upon the cancer, or the result of some secondary interference. With the cancer mice at our disposal it is hoped that we shall be able to obtain positive results bearing on the question of immunity and serum diagnosis, to which it is proposed to devote the special attention of this Laboratory during the forthcoming year.

STUDIES IN CANCER METABOLISM.

By G. H. A. Clowes, Ph. D., W. S. Frisbie, Ph. B., and H. H. Glosser, M. D.

A review of the literature published in the course of the last fifty years on the equilibrium of nitrogenous bodies and salts in cases of impaired metabolism makes it apparent that the most divergent views are held on this subject by different investigators. Marked disturbances in the secretion of urea and chlorides in cachectic cancer cases were first observed by Vogel, Rommelaire, Jacoby and others, but these early investigators made very little attempt to carry out strict analyses on the food stuffs consumed in comparison with the waste products excreted. Müller and Klemperer (to whose publications a detailed reference will be made later) came to the conclusion that under the influence of toxic bodies produced from tumors a loss of nitrogen was suffered by the body, while Moraczewski in the majority of his cases finds a retention of nitrogen in the system, the amount contained in the food being in excess of that secreted in the urine and fæces. Similar differences of opinion are to be noted in the works of Müller, Laudenheimer, Schöpp, Bouveret, Böhne and Moraczewski on the retention or secretion of chlorides. A considerable increase in the proportion of sodium chloride in the blood of certain cancer cases has been observed by Moraczewski, and it has even been claimed that greatly increased quantities of chlorides are retained by certain organs of the body, especially in those cases in which an œdematous condition is to be observed. In many of the cases investigated disturbances in the equilibrium of salts were found to be associated with an impaired secreting function of the kidney, which would make a clear differentiation between cause and effect extremely difficult. Great advances have been made in physical and physiological chemistry since the publication of the work of these early investigators. The laws of osmosis governing the equilibrium between soluble salts, the body fluids and the cells render it extremely improbable that large quantities of sodium chloride, for example, could be retained in the system, as has been claimed by some writers, without the retention of a corresponding quantity of water necessary to maintain, at any rate to a certain extent, the osmotic equilibrium, which would cause an increase in body weight. The ability of certain electrolytes to aid in the removal of products of suboxidation from the system, has long been recognized and more recently the studies of Köppe, Hamburger, Gerber, etc., on the metabolism of blood cells have demonstrated the significant role played by chlorine ions in the removal of products of oxidation from the cell. It is perfectly conceivable that toxic bodies produced from a tumor might seriously interfere with normal osmosis; also that the abnormal products produced through the disintegration of cells and the influence of toxins might prevent to a certain extent the dissociation of salts, thus interfering with their normal function and at the same time reducing the osmotic tension which they exert and lead to their retention in larger quantities in the system, such a process being accumulative in its action. Loeb, Matthews, Calkins, etc., have shown how significant is the role played by electrolytes in the stimulation of individual cells, and the possible influence of disturbances in osmotic equilibrium of the cell leading to its proliferation can scarcely be ignored.

In this paper it is proposed, in the first place, to summarize the existing literature bearing on the problem of nitrogen and chlorine metabolism in cancer as compared with the normal, and subsequently to record some results having a bearing on this subject obtained in this Laboratory in the course of the last two years. Whilst our main object in carrying out these experiments has been to find some means of improving the nutrition of cases in which tumors have directly or indirectly interfered with the proper assimilation of food, and also to study the effects exerted by various salts and chemical bodies when taken in conjunction with the food or on direct injection, the exact analyses carried out from day to day on the diet, urine and fæces, and frequently on the blood, enables us to throw some light on the discrepancies observed in the literature and to arrive at definite conclusions which will be referred to at a later stage.

In reviewing the early literature one of the things that first impresses itself on one's mind is the absence of accurate data regarding the diet, the frequent disregard of the loss of nitrogen and chlorine in the fæces and the failure to record variations in the weight of patients. It should be possible to obtain a more or less accurate balance between the increase in weight of the tumor, the loss of body proteid, the formation of œdematous fluids and all such factors, when considered in conjunction, with the differences between intake and output of nitrogenous bodies and salts in a given period of time. In work of this nature the sources of experimental error, especially in the hospitals, are, as Müller points out, extremely numerous. Our own experience has taught us that unless the intelligent cooperation of a patient is obtained, or at least of all those immediately concerned with the charge of the case, it is perfectly useless to look for even moderately reliable results, however accurate the work in the laboratory may be. It is very probable that careless patients, especially those capable of moving freely about the wards, have deceived those who were interested in studying their metabolism, not only as regards their diet but also with reference to the twenty-four-hour secretion of urine, a fact which might well explain the contradictory results obtained by different authorities. One surprising source of error frequently met with in the literature is to be found in the change from a solid to a liquid diet. Milk without any addition of chlorides contains considerably less chlorine in proportion to the nitrogen than does the normal diet, consequently a diminished secretion of chlorides would be observed in the urine, and is not necessarily in any sense attributable to the influence of a tumor or other pathological cause.

Vogel, who was the first to investigate quantitatively the constitutents of the urine of a case of cancer of the liver, observed a daily secretion of urea under 8 grams and assumed that an actual retention of proteids took place in the blood in excess of those consumed in order to provide the materials required by the tumor cells in their development. Since, however, no records of the diet, of the nitrogen content of the stools or the body weight of the patient are recorded, this conclusion is scarcely justifiable. Rommelaire, in cases of cancer of the stomach, and Gregoire confirmed Vogel's observation of a greatly diminished secretion of urea, but these investigators seem also to have overlooked the probable diminution of proteids in the diet, and whilst reference is made to diarrhœa, no account seems to have been taken of the loss of nitrogen by that source. Robin expressed himself as opposed to the views held by the above-named writers, claiming that in well-nourished cancer cases the urea secretion was normal, and assumed that the diminution of nitrogen in the urea was simply to be attributed to a diminution of the quantity of proteid in the diet. Dujardin-Beaumetz supports Robin, finding in certain cancer cases high urea secretion, reaching 17 and 25 grams per diem, which he considers may be attributed to the influence of toxins secreted by the tumor, whilst certain nonmalignant tumors show results as low as 4 grams per diem.

The first work of this nature which can be said to have any real value was published by Jacoby in 1887. This author compared the daily nitrogen excretion of a normal individual with that of a case of carcinoma of the liver associated with jaundice, both cases receiving identically the same diet. He succeeded in demonstrating that whilst a normal individual could be made to gain weight and secrete less than 12 grams of nitrogen daily, the cancer case would lose weight and as much as 18 grams nitrogen would be eliminated in the urine.

The next paper on cancer metabolism possessed of any considerable value was published in 1889 by Friedrich Müller, who made an exhaustive study of the proteid equilibrium in a series of nine cancer cases, in some of which nutrition was mechanically interfered with by the tumor, and compared the results so obtained with experiments previously carried out by himself and other investigators (Senator, Monk and Zunz) on starving individuals. The conclusions arrived at by Müller are extremely interesting. He succeeded in demonstrating that in all his cases in spite of a rapid development of the tumor more or less loss of nitrogen was suffered by the body as a whole when the heavy loss of that substance in the fæces was taken into account. He also proved that this loss was more or less proportionate to the loss of body weight when due allowance was made for the formation of œdematous fluid. He further demonstrated that in those cases in which the nutrition of the patient was seriously disturbed by the developing tumor in such a manner that the loss of nitrogen by the fæces was practically equal to that contained in the food, the condition of starvation thus established was very comparable with that obtained in his experiments on starving normal individuals. In studying the influence of toxins produced by the tumor, Müller made one very valuable observation to the effect that whilst the average loss per kilo of body weight per diem was in the case of a series of starving healthy individuals only .103 grams nitrogen, that of four cachectic cancer cases amounted to .175, .225, .166 and .146 respectively. He also noted that even in one case in which the appetite of the individual under experiment was considerable, it was still found impossible actually to maintain the nitrogen equilibrium, although the amount of nitrogenous material assimilated was far in excess of that which would have been required by a normal individual of the same weight. He notes similar results in a case of typhoid during the early period of the disease and whilst the patient was suffering from toxic symptoms, the exactly reverse results being obtained during the period of convalescence. Müller concludes that in all cases of cancer the destruction of proteid suffered by the body owing to faulty nutrition and toxic effects is greater than the increase in proteid of the growing tissue of the tumor, and that consequently a loss of nitrogen is always suffered by the system. He attributes the coma of carcinoma to auto-intoxication through the products of metabolism which have not been removed owing to faulty functioning of kidneys affected by toxins. It is only to be regretted that this extremely accurate worker failed to take account of the chlorides present in the diet in order that they might be compared with those of the urine and fæces. In his analyses of urine the quantity of chloride was frequently extremely low, and he concluded without much ground that this signified the destruction of body proteid rather than circulating proteid. Sticker and Huebner observed a much higher secretion of sodium chloride in nonulcerating carcinoma than was the case in ulcerating carcinoma of the pylorus. G. Klemperer, who carried out fairly exact metabolism experiments on seven cancer cases, confirms the original observation of Jacoby, that when normal individuals and cancer cases are submitted to the same conditions of experiment, the former may gain whilst the latter lose in body proteid, a difference in nitrogen content of the food and excreta as high as 7.5 grams daily being observed. Huebner considers that the diminution in the alkalinity of the blood, and the fatty degeneration of organs, associated with increased tissue destruction, may be looked upon as evidence that carcinoma is an intoxication disease, and that the coma associated with the last stages may be attributed to the action of the poison derived from the tumor circulating in the blood and acting upon the central nervous system. Bouveret attempted to differentiate between diseases associated with hyper-secretion of hydrochloric acid in the stomach and cancer of the stomach by means of the secretions of chlorine in the urine, claiming an increased excretion associated with diminished secretion of hydrochloric in the stomach. This finding was not in agreement with that of Stroh, who observed a diminution in the chlorides secreted in both cases. Laudenheimer, experimenting on five cancer cases with reference to the chloride equilibrium, obtained in two cases a secretion of chlorides comparable with the consumption and in two other cases a marked diminution of the secretion of sodium chloride in comparison with the consumption. This author concludes that the disturbances in chloride equilibrium are the result of nephritic or circulatory retention of water and salts. Schöpp in a couple of papers claims that whilst the excretion both of chlorine and nitrogen frequently falls below the amount assimilated in cancer cases, the difference may generally be attributed to ulceration of the tumor, the secretion containing considerable quantities of sodium chloride. In a case of cancer of the stomach and one of breast carcinoma, equilibrium was maintained in the absence of ulceration.

From 1895 to 1897 a series of important papers were published by Moraczewski on the blood and urine in cancer, on metabolism in carcinoma and chlorisis, and subsequently on leucæmia and pseudo-leucæmia. In the first paper on the chlorine and phosphorus content of the blood and urine in cancer, after referring to the literature, the author estimated phosphorus, chlorine and nitrogen in the blood and urine of cases of carcinoma, anæmia and other pathological conditions. He found a reduction in the phosphorus content of the blood from the normal of .1 per cent to .07 and .04; chlorine, on the other hand, was high, amounting to as much as 4 per cent of the dried substance. He remarked further that as the chlorine in the blood increased the chlorine in the urine decreased in cases of carcinoma, but no particular reference was made to the condition of the kidneys. He considered that the nitrogen content was also increased in proportion corresponding with the increase of chlorine in cancer but not in anæmia. Moraczewski's work on cancer metabolism published in 1897, eight years later than that of Müller, gives results which are in no sense in accord with those obtained by the latter investigator. Whilst Müller observed a heavy loss of nitrogen in a long series of cases, Moraczewski found that large quantities both of nitrogen and chlorine were retained by the system, and at the best an approximate equilibrium was established. In none of his cases was a loss of nitrogen recorded over any extended period. The amount of nitrogen and sodium chloride retained in the system in a short space of time was frequently vastly in excess of the amount which would correspond with the changes in weight of the patient. In fact, in certain cases it is apparent that there must have been some source of experimental error, the quantities secreted being so far below those assimilated that either the patient must have gained ten or twenty kilo instead of, as was actually the case, undergoing very slight changes in weight, or chlorides must have been deposited in the tissues, which is practically impossible. Were it not for these discrepancies this work of Moraczewski's would be a most valuable contribution to the study of cancer metabolism. This author observed the proportions of nitrogen, chlorine, phosphorus and calcium in the twenty-four-hour urine whilst subjecting the patient to the influence of various salts, the diet being maintained on as uniform a basis as possible. He concluded that in cancer large doses of sodium chloride or calcium phosphate may be used to effect a saving of proteid whilst they stimulate the excretion of chlorides. He failed, however, to take account of the probable retention of waste nitrogen products in the œdematous fluids resulting from the presence of large quantities of these salts. He found that silver nitrate given in small doses increased the secretion of nitrogen and phosphorus and decreased that of chlorine and calcium, chlorides being retained as might be expected. Silver nitrate, however, produced diarrhœa and other acute symptoms in some of the cases to such an extent as to be discontinued. More especially was this effect to be noticed in anæmic cases. He concluded that the retention of chlorides is increased with the increase of anæmia, which is in accord with the supposition that such a retention of salts might merely represent the dilution of blood or body fluids which takes place under these conditions. He also found that phosphorus is retained in conjunction with chlorides in spite of the fact that phosphorus is present in the blood in proportions which are frequently the inverse of those of chlorine; also that the secretion of calcium is inversely proportional to that of phosphorus, and that a larger amount of calcium is retained in slightly anæmic conditions. In one case, for example, the addition of ten grams of sodium chloride daily to the food led, as would be expected, to an increase in the retention of chlorine and nitrogen, whilst the entire elimination of chlorides from the diet and the addition of ten grams of calcium phosphate led to a considerable chlorine loss. On the other hand, .1 gram of silver nitrate resulted in what was practically a balance of the proportions of nitrogen and chlorine, a much larger amount of chlorides being retained than during the treatment with calcium phosphate. Ten grams of sodium phosphate led to a retention of nitrogen and calcium, chlorine remaining about normal. Five grams of sodium chloride and 5 grams of potassium chloride led to a retention of nitrogen and chlorine and a proportionate loss of calcium and phosphorus. On comparing cancer cases with those of chlorosis he concluded that the latter are capable of taking much larger doses of potassium chloride than the former without noticeable toxic effect. Whilst an immense number of results were recorded in this paper, their failure to balance and a want of agreement in some cases detracts from their value.

Moraczewski also published a series of experiments on the chlorine, nitrogen, phosphorus and calcium equilibrium in a case of leucæmia and the data which he obtained extending over a period of six weeks, whilst the patient was subjected to a variety of different treatments, would indicate that a very considerable retention of nitrogen and chlorine in the proportions respectively of 40 and 30 per cent of the diet had taken place, a result which is most remarkable when it is borne in mind that in spite of the apparently rapid development of the leucæmic tumor no great gain in weight had been experienced by the patient.

Böhne found in a marked cachectic cancer case an average retention of 3 or 4 grams chloride daily for the space of four weeks, and claimed to have observed on post-mortem examination of the liver a sodium chloride content four times as great as that in the livers of three other patients. His observation has been supported by that of Achard and Loeper, who claim that the salt content of the muscle tissue of œdematous cases (non-cancerous) in which the chlorides are strongly retained, can be as high as 4.1 to 5.6 grams per 1000 in comparison with the normal, which is 1.6 to 2.8 grams per 1000; also that the brain substance, which normally contains 1.1 grams per 1000, may under conditions of abnormal retention give figures as high as 4.3 per 1000.

Cetti, in a publication concerned principally with the relative proportions of nitrogen constituents in the urine, found in two cases a secretion of nitrogen in excess of the consumption; in two other cases an ability to establish a fair equilibrium. He concluded that an increase in the proportion of extractive nitrogen is always associated with a decrease in the proportion of urea nitrogen as the disease progresses. He simply observed with reference to the chlorides that the total secretion was low, except in the case of one patient, who was receiving a full, normal mixed diet.

Koziczkowsky, in a valuable paper on the equilibrium of chlorides, refers to the frequently observed diminution in the excretion of chlorides in fevers (with the exception of malaria) and in carcinoma. The experiments recorded in this paper are directed principally to the solution of the problem as to whether chlorides, and to a certain extent other soluble salts, play a direct or indirect role in nephritis and œdema. He concludes that the retention or secretion of chlorides does not follow the same laws as those which govern the removal from the body of the products of metabolism, urea and other nitrogenous bodies, phosphates, sulphates, etc., but fails to bring very much evidence to bear on the question as to whether the retention of chlorides is due to their association in the system with katabolic products, or to disturbances in the osmotic equilibrium and mechanical forces of the body, and the ready diffusion through cell membranes, factors which might prevent the normal removal of salt. Engelman, in a communication on the osmotic pressure of body fluids, records results considerably above the normal in certain cachectic cases, but such results are generally attributable to heart and kidney insufficiency.

Braunstein, in a publication* dealing with the secretion of chlorides, phosphates, nitrogen and ammonia in carcinoma, after reviewing the literature, gives the results of a series of analyses carried out on the urine of 10 cancer cases, in five of which an exact record has been kept of the nitrogen and chlorine of the diet. In three of the five cases a loss of nitrogen was to be observed. In one case of carcinoma of the œsophagus the influence of the tumor on the destruction of proteid was very marked, the average daily loss for a period of four days being 10.6 grams nitrogen. In this case the chlorides were extremely low, both in the diet and in the excreta, no great difference being observed. Numerous experiments carried out on healthy individuals in a state of inanition show a loss of only three to five grams nitrogen daily, consequently any such loss as that recorded in the case referred to must be due to toxic action leading to an abnormal destruction of proteid. Braunstein failed to find any considerable differences between the salt content of the diet and the excreta, in certain cases the latter even exceeding the former. His experiments with diuretin led him to conclude that the sodium chloride secretion was directly proportional to the urine secretion, and that the accumulation of chlorides observed in certain cases was simply attributable to the failure of the kidneys to remove water, and with it the salts held in solution. He further states that in cancer an increase rather than a decrease of the secretion of chlorine in the urine may be looked for provided the kidney function is satisfactorily performed.

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*This publication was received in June, 1904, after the completion and shortly before the publication of this paper.

To summarize the literature on this subject, it may be said that widely differing results have been obtained by different authors, as regards the nitrogen and chlorine metabolic equilibrium of cancer cases. Whilst on the one hand it is claimed that nitrogen is retained in the system as a result of the tumor development and the demand created thereby for proteids, on the other hand it is shown that in spite of the tumor growth the nitrogen loss suffered by the system due to the destructive influence of toxins on the body proteids is very considerable except in those cases in which a marked œdema has developed, and in which the failure of the kidneys to function has led to an abnormal retention of waste nitrogen. Whilst numerous authors claim to have observed a diminution in the total amount of nitrogen and chlorine of the urine, especially in the last stages of cancer, few of the early writers regarded sufficiently diminutions in the proportion of chlorides in the diet, especially in those cases in which patients were no longer capable of assimilating solid food. Analyses of organ and body fluids showing an extraordinarily high content of chlorides, and records of the retention of enormous quantities of salt in the system far in excess of the amounts which would correspond with the observed variations of body weight and the laws of osmosis, lead us to suspect some source of experimental error, especially in view of the great difficulty in such work of properly controlling the individual submitted to experiment.

The possible importance of the chlorides in the establishment of metabolic equilibrium is more readily appreciated at the present day than was possible at the time at which most of the experiments referred to above were carried out. The intervention of modern physico-chemical theories of solution in physiological problems has demonstrated the important role played by individual ions and the dependence of any such influence upon the concentration and degree of dissociation of the compound from which they are derived. It has long been known that in fevers and other morbid conditions the equilibrium of the chlorides in the system is materially interfered with. This action may be due in part to mechanical causes brought about indirectly by the action of toxins on the cells of the heart, kidneys and other organs more especially concerned with the balancing of pressure, and consequently to a certain extent of diffusion and osmosis in the system. It may also be attributable to the action of toxins and of toxic products of faulty metabolism capable of clogging, so to speak, the normal functions of the salts in the system by preventing their proper dissociation into ions. The effect of toxins and foreign bodies upon the protoplasm of those cells of the stomach and kidneys, for example, which are capable of exerting a so-called vital secretory action on chlorides, etc. (not necessarily, so far as our present knowledge is concerned, in direct accord with the laws of osmosis), resulting in an interference with its normal function, its degree of viscosity and permeability, is a problem still open to investigation. These and many other possibilities must eventually be carefully considered, but bearing in mind the innumerable observations in cancer cases of interference with the secretions of the stomach, of increase in the chlorides and diminution in the alkalinity of the blood, of increase in the osmotic pressure of blood (Engelmann) and tissues (Hemmeter), of changes in proportion of salts in the tissues, of the production of œdematous fluids and of the diminution in the secretion of chlorides and variation from the normal rise and fall of chlorine secretion in the course of the day (Alb. Müller), it seems a matter of the first importance to throw as much light as possible upon the nature of the interference exerted in such cases, especially in view of the recent work on blood showing the role played by chlorine ions in the processes of oxidation and removal of waste products from the cells. The further possibility of variation in the proportion of potassium, sodium and calcium and other ions having a direct effect upon the proliferative tendencies of certain cells (referred to above) gives an added importance to the frequently ignored problem of salt equilibrium.

The main objects of our investigation have been as follows:

(1) To determine whether the change in weight of cancer patients was associated with a corresponding loss or gain of nitrogen by the system, and if so to what extent such results compared with those obtained from the study of normal individuals.

(2) To compare the chlorine of the food with that of the excreta with a view to discovering whether any absorption of chlorine by the body takes place, and if so to what extent.

(3) To determine whether the balance of chlorine retained in the system obtained by deducting the amount present in the excreta from that present in the diet possesses any direct relationship to the supposed increase in the chlorides of the blood and the formation of œdematous deposits; or whether the assumption of a diminished chloride dissociation can be made to account for the phenomena observed.

(4) To study the effect of adding or totally excluding chlorides from the diet as compared with the effect of such a treatment upon a normal individual.

The analytical methods employed were nitrogen by the Kjeldahl process, sodium chloride by the Salkowski or Moraczewski method, ammonia by the procedure of Schlösing, uric acid by the Hopkins method, phosphates by titrating with uranium nitrate solution and other constituents of the urine as described in the text.

METABOLISM EXPERIMENTS.

CASE I. NORMAL INDIVIDUAL.

The absence of accurate data in the literature regarding the influence of varying proportions of chlorides and other salts upon the metabolism of normal individuals rendered it advisable to carry out a series of experiments on one of ourselves which might afford a basis for comparison with some experiments made on individuals suffering from cancer. Dr. G.; physician; age 32; weight 66 kilo; health normal, received for two separate periods (one of 13 days, the other of 9 days) a diet in which the exact amounts of nitrogen and chlorine were carefully determined. In addition variations in body weight were recorded and the total volume, specific gravity, nitrogen, chlorine, phosphorus, uric acid and ammonia of the urine and the nitrogen and chlorine of the fæces were estimated. For the first five days a comparison of the nitrogen content of the food and excreta showed that a state of equilibrium might most readily be maintained on an average consumption of proteid containing from 13 to 14 grams nitrogen per diem. For the remainder of the first period and the second period considerable variations in nitrogen and chlorine content of food were accompanied by corresponding variations in the proportions of those substances in the excreta. The tendency to establish a state of osmotic equilibrium may most readily be recognized by drawing curves representing the variations in body weight, nitrogen and chlorine of the food and nitrogen, chlorine, uric acid and ammonia of the urine recorded in Table I. The weight of the body was found to be far less affected by the variations in nitrogen than by those in chlorides. Any large increase in the amount of proteid food consumed in excess of 14 grams nitrogen was apparently only partially removed by the urine, and whilst an increased amount was eliminated by means of the fæces, a considerable portion was apparently retained in the system. For the last two days of the first period a considerable reduction of the proteid of the diet (showing respectively 9.1 grams and 5.1 grams nitrogen in the food), led to interesting results. Whilst the reduction of the food from about 13½ grams to 9 grams nitrogen led to a fall of the total nitrogen of urine and fæces from about 13½ to 11.3, a reduction of the total nitrogen of the food to 5.1 in the last day was accompanied by a total nitrogen excretion of 9.3 grams. An examination of these figures shows that the reduction in the nitrogen of the food is accompanied by a proportionate loss in nitrogen on the part of

TABLE I.

First Period,

DATE.	Nitrogen in food.	Nitrogen in urine and fæces.	Sodium chloride in food.	Sodium chloride in urine and fæces.	Body weight in kilgms.	Uric acid.	Ammonia.
January.							
24–25	14.0	14.1	......	......	65.9	.58	......
25–26	15.3	15.0	......	......	65.1	......	......
27–28	12.4	13.5	7.9	8.1	65.4	.33	.87
28–29	15.5	13.7	8.1	7.9	66.1	.34	.80
29–30	13.9	15.9	27.6	20.0	66.4	.74	.63
30–31	13.9	14.2	23.0	23.6	66.5	.51	.81
31–1	17.6	14.6	12.1	17.6	66.1	.46	.76
1–2	13.0	13.1	2.0	9.5	65.1	.33	.78
2–3	9.1	11.3	1.3	3.8	64.3	.36	.60
3–4	5.1	9.2	9.3	5.4	64.7	.46	.56
Second Period.							
March.							
30–31	12.2	10.7	......	......	66.7	.48	......
31–1	15.4	17.6	32.2	26.5	66.7	.67	.91
1–2	12.8	13.2	30.3	27.8	67.5	.52	.83
2–3	14.0	14.1	7.8	12.8	67.0	.43	.97
3–4	11.4	13.9	6.5	9.7	66.7	.46	.76
4–5	11.6	13.9	4.1	6.8	66.4	.47	.84
5–6	12.1	12.8	6.6	6.3	65.8	.38	.69
6–7	8.2	11.3	13.7	10.6	65.7	.43	.82
7–8	6.6	11.3	3.8	7.6	66.0	.35	.57

the body, but that the loss in question is not equal to the difference between the nitrogen of the food for the day in question and the normal, but about one-half of that quantity. During the greater portion of the second period the nitrogen of the food was maintained on a fairly low level. For the first four days an average consumption of about 13.6 grams nitrogen was accompanied by a corresponding loss of 13.8. The following three days on which the nitrogen of the food was still further diminished to an average of 11.7 showed an appreciable loss of body proteid, the average of the urine+fæces being 13.5. On the last two days an average of 7.4 nitrogen in the food was associated with an excretion of 11.3 grams nitrogen, or a difference of nearly 4 grams derived from body tissue. In this case, as in the first period, it will be observed that the loss of body proteid is not equal to the difference between the nitrogen of the food consumed in the period in question and the normal average amount for a corresponding period, but represents a certain proportion of that amount, in this case rather more than half.

Owing to the difficulty of carrying such experiments further it was not possible to test the effect of still further diminishing the proteids of the food or entirely removing them from the diet for the space of another day. The figures obtained by producing curves would indicate that the amount of nitrogen lost daily in a state of absolute starvation would be from 6 to 7 grams (or .1 gram per kilo) thus exceeding the figures 3 to 5 grams given by Klemperer and Blumenthal. They would on the other hand be lower than those obtained on Cetti by Senator, Zunz, etc., which are considered to have been abnormally high, owing to the toxic influence of the tuberculosis from which it was discovered that Cetti was suffering.

These normal experiments and a second series on a case of cardio-spasmus, which will be referred to later, justify the assumption that the daily loss of an individual weighing 66 kilos would be from 6 to 7 grams in a state of absolute starvation provided no toxic influences were concerned; consequently the loss of nitrogen per kilo of body weight in excess of .1 gram per diem may be attributed to the influence of toxic agencies, a point which will be referred to at a later stage in considering the metabolism of cachectic individuals.

Under normal conditions the sodium chloride of the food and of the urine lay apparently between 7.5 and 8 grams per diem. On raising the total sodium chloride of the diet in the course of one day from 8 grams to 27.6 only 19.3 was excreted from the kidneys which, with .7 in the fæces, gives a total of 20.0, thus showing a gain of 7.6 grams by the body. The following day the consumption of 22.99 grams sodium chloride was accompanied by a loss of 22.8 in the urine and .7 in the fæces, giving a total of 23.6 for the excreta. A practical balance was thus established on this day. On the following day, when 12 grams only were consumed, a total of 17.6 was eliminated, the body thus tending to recover its normal chlorine equilibrium, a result which was completely effected on the following day when, starving the patient of chlorides, only two grams being introduced into the diet, as much as 9.5 grams was still excreted. A still further reduction in the chlorides of the food to 1.3 on the following day was accompanied by a loss of 3.5 in the urine, or a total of 3.78 in urine and fæces, thus showing a still further loss on the part of the body of salt. The following day ended the first series of experiments with an increase in the consumption of salt to the normal figure, about 9.2 grams, and a loss of only 5.3, showing a tendency on the part of the system to retain chlorides in order to replace those which had been secreted during the previous period of chlorine starvation. Very similar results are to be observed in the second period, 32.2 and 30.3 grams in the diet the first two days being associated with a loss of 26.5 and 27.8, showing a retention of 5.7 and 2.5 grams, or a total of 8.2. On the two following days a reduction of the chlorides of the diet to quantities slightly below the normal was accompanied by a loss of 5 grams and 3.2 grams respectively, or a total of 8.2 grams, thus showing an exact balance of chlorides for the first four days. On the fifth day a reduction of the sodium chloride of the diet to 4.1 grams was associated with a loss of 6.8 grams, thus showing a loss of salts on the part of the body. The same interesting tendency to maintain a balance is to be noted to the extreme end of the series, the sum totals of intake and output of chlorides being for the entire period almost identical. The chlorides retained in the fæces normally amounted to .2 grams daily, but on those days on which large quantities were consumed an increase to .71 and .77 respectively was noted. In view of a recent publication to the effect that the chlorides only pass by way of the fæces after 24 hours, it is interesting to note that the almost full effect of the large doses of chlorides were observed in the space of the first 24 hours after their administration, and that without any accompaniment of diarrhœa. The effect of large consumption of NaCl on the nitrogen excretion is marked, the maximum loss of nitrogen in each period being associated with the maximum consumption and excretion of chlorides.

The body weight was found to vary directly with the amount of chlorides consumed, or more strictly, those retained in the system. In the first period the maximum of weight, 66.4 to 66.5 kilos, was observed in the course of the two days on which the largest amounts of sodium chloride were consumed and 7½ grams retained in the body. The gradual diminution of the amount of chlorides in the diet, with a gradual increase of the proportion of chlorides excreted until the latter were considerably in excess of the former, led to an exactly corresponding decrease in the weight of the individual. The minimum weight observed in the series of experiments, 64.3 kilos, was reached on the day of maximum chloride starvation, the day on which 1.3 grams only of sodium chloride was consumed in the food and 3.8 excreted, following the day on which 2 grams in the food had been accompanied by a loss of 9.5 grams. The total loss of weight between the maximum and minimum points, slightly in excess of 2 kilos, was accompanied by a loss of chlorides amounting to more than 15 grams, which would fully account for that difference in weight, assuming that a .75 to .8 per cent sodium chloride solution were isotonic with the serum. The following day a considerable increase in the salt of the diet was accompanied, as would be expected, by a retention of that substance in the system previously impoverished and consequently an increase in weight of the body was observed, although at the moment a considerable diminution in proteid in the diet accompanied by a loss of nitrogen would normally have led to a loss of weight.

In the second period the balance is even more exact. At first the retention of 5 or 6 grams sodium chloride is accompanied by an increase in weight of .8 of a kilo to a maximum of 67.5. Subsequently a fall in weight to 65.8 was accompanied by a loss of sodium chloride of nearly 11 grams. In this period also the actual loss of nitrogen on the part of the body produced an insignificant effect upon the body weight as compared with the variations in chlorides. A comparison of the volumes of water ingested and excreted shows on the average a preponderance of the former over the latter by 500 or 600 c.c. On those days on which large quantities of salt were consumed the volume of fluid ingested was about two liters and that excreted about 1300 c.c., the big secretion of sodium chloride being accompanied by a corresponding increase in total volume of urine, the specific gravity never rising above 1.023 to 1.022. On only one day in the series was the total volume of the urine in excess of the volume of water ingested, the day on which chlorides being reduced to a minimum in the diet, a large excess of salt in the system was removed in the urine.

It will thus be seen that the data bearing on body weight and intake and output of water correspond exactly with the variations in the proportions of chlorides of the diet and excreta. An investigation of the chloride figures makes it appear probable that a period of 48 hours frequently elapses before chloride equilibrium is completely established in the system, but such an equilibrium is effected very readily in contrast to the pathological cases referred to subsequently.

Effect of variations in the proportion of chlorides upon other constituents of the urine.—On those days on which the normal amount, about 8 grams of sodium chloride, was consumed in the food and excreted in the urine, the nitrogen of the urine was about 12.5, the phosphates, estimated as P_2O_5 about 2.5, and the uric acid .33 to .34 grams, the ammonia about .6 grams. A great increase in the amount of salt in the diet led to a great increase in the total volume of the urine, a slight increase in the total nitrogen excreted, an increase in the phosphates and a very large increase in the amount of uric acid, with a slight decrease in the ammonia. The uric acid results are most instructive. Two days of a normal salt diet with a uric acid secretion of .32 and .34 grams were followed by three days on which an abnormal amount of salt was consumed and gradually excreted by the kidneys, on which days respectively .74, .51 and .46 grams of uric acid, giving an average of .57 grams per diem, were removed. It will thus be seen that during the period of large salt consumption and increased excretion of water and chlorides either an increased destruction of the nucleus of cells was brought about, leading to an increase in the excretion of uric acid, or a more complete removal of the latter substance from the circulation was effected, owing to a great increase in the amount of water and salts. This latter theory is in accord with the results published by one of us on the freezing point of the urine, which indicate a probable relationship between the excretion of small ions and of large complexes such as uric acid. Following the uric acid table further, it is interesting to note that in the next two days of chlorine starvation the quantity of sodium chloride excreted adjusts itself to the normal and is accompanied by normal amounts of uric acid .33 and .36 respectively. On the last day where the factor of starvation has to be considered an increase in the amount of chlorides and partial starvation led to an increase in the amount of uric acid, .46 grams being removed. Similar results were recorded in the second period.

The excretion of ammonia appears to be much less affected than is that of uric acid by the variations in proportions of sodium chloride, but the first day on which large quantities of salt were excreted shows a diminution in the amount of ammonia, and it may be said that, generally speaking, a slight fall in the ammonia in proportion to the total nitrogen appeared to take place when large doses of salt were employed.

Throughout these series of experiments and in spite of large variations of chlorides the relationship of the freezing point to the specific gravity was found to be far more constant than would be anticipated.*

Analyses of the nitrogen and sodium chloride content of the blood were made on April 1st at the point of maximum salt assimilation and on April 5th at a stage of salt starvation:

	Nitrogen.	Sodium Chloride.
April 1st	3.01	.402
April 5th	3.43	.427

From which it will be seen that whilst the nitrogen is greater on April 5th, the proportion of sodium chloride to nitrogen is higher on April 1st.

The effect of chlorides tending to produce œdema when administered in large doses.—The increase in body weight to a maximum under the influence of large quantities of sodium chloride and its fall below the normal on reducing the salt in the diet to a minimum whilst the proteid consumption was maintained at a fairly constant level and the nitrogen equilibrium of the system underwent practically no change, has been referred to in the discussion of the body weight and chloride balance. It was further shown that the loss of chlorides suffered by the body between the maximum and minimum weights corresponded fairly well with the difference in weight, on the assumption that a salt solution almost isotonic with the blood serum was being removed from the body. It was also noted that the variations produced by changes in the chlorides were so much more marked than those brought about by reduction or increase of the nitrogen as to entirely mask the effects of the latter on the body weight.

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*See "The relationship between the freezing point depression and specific gravity of urine, under varying conditions of metabolism, and its clinical value in the estimation of sugar and albumin," by G. H. A. Clowes, American Journal of Physiology, Vol. IX, July 1, 1903.

It is interesting to note the space of time required by the body of a healthy individual to bring about a chloride equilibrium, the figures recorded showing that this may be accomplished in from 24 to 48 hours, a point which will be referred to subsequently in carrying out some experiments on œdematous pathological cases.

*CASE II. CARCINOMA OF THE PYLORUS.

J. W.; age 75; weight 51 kilo.; widower.—Gives no definite history of duration of disease. Has for months past experienced considerable difficulty in taking food. Is very much emaciated and very cachectic and is not sufficiently intelligent to give a reliable account of himself. Examination shows prominent tumor on inspection in the epigastrium, which on palpation is hard, immovable and seems to involve not only the pylorus but the tissues about it. Patient is very feeble.

Death occurred on April 2, 1903, and the autopsy revealed the following conditions: On opening the abdomen about 300c.c. of turbid fluid was found in the region of the stomach and spleen. No general peritonitis was observed. A large tumor mass, irregular in shape, involving most of the pancreas, the entire pyloric end of the stomach and one-third of its greater curvature, was found. The size of the mass was about that of two fists. Neither duodenum, liver nor any other abdominal organ was involved. On section the pylorus showed a very small opening into the duodenum and a surface as large as a man's hand of tumor tissue ulcerating and necrotic. Pathological examination confirms diagnosis of carcinoma of pylorus with metastases involving the pancreas.

For a period of twelve weeks analyses were carried out on the complete 24-hour specimens of urine and fæces. For the eight weeks immediately preceding death the total nitrogen and chlorine in the food were estimated from day to day. During this latter period the patient was for the most part on a liquid diet, which considerably facilitated compilation of data. The principal objects to be gained by this series of experiments were (1) a comparison of the nitrogen and chlorine of the food and excreta over an extensive period immediately previous to the decease of the patient; (2) the influence of varying proportions of sodium chloride in the diet; (3) a comparison of the relative

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*We are indebted to Dr. Matzinger of the Laboratory staff for the histories and autopsies on this and subsequent cases, III, IV, V and VI.

proportions of various nitrogenous constituents, phosporus, chlorine, etc., in the urine, under the varying conditions that obtain at various stages in the development of the disease.

1. *Nitrogen and Chlorine Equilibrium.*

The results obtained have been tabulated in weekly periods showing the intake and output of nitrogen and chlorides as Na Cl and the gain or loss of these substances experienced by the body for each period (see Table II). An examination of the table shows that for the first two weeks the body was practically in a state of nitrogen and chlorine equilibrium, a total of 91.4 grams of nitrogen being assimilated and the same amount excreted, whilst 26.0 grams of chloride in the food corresponded fairly well with 25.0 in the urine and fæces without any appreciable loss being experienced in body weight. During the succeeding week, from the 18th to the 26th, 14 grams of nitrogen were lost and 2.8 grams of chlorine retained by the body, the condition of the patient showing a marked falling off. In the three succeeding weeks the body suffered a steady loss of nitrogen and exhibited an ever-increasing tendency to retain chlorine, till finally, in the 14-day period immediately preceding death, whilst the nitrogen loss was 33.7 grams, the retention of sodium chloride amounted to as large a quantity as 22.8 grams, only 3.5 grams being eliminated in the urine out of a total of 26.3 contained in the food. That is to say, over 80 per cent of the chlorine assimilated with the food remained in the

TABLE II.

	Nitrogen in food.	Nitrogen in urine and fæces.	Loss or gain, nitrogen.	Chlorine in food.	Chlorine in urine and fæces.	Loss or gain, chlorine.
Feb. 4–11........	45.0	49.0	− 4.0	11.8	11.7	+ .1
11–18........	46.4	42.4	+ 4.0	14.2	13.3	+ .9
18–26........	36.5	50.6	−14.1	13.0	10.2	+ 2.8
26–Mar. 5....	32.9	40.2	− 7.3	8.8	4.7	+ 4.1
Mar. 5–12........	45.9	50.4	− 4.5	12.2	4.0	+ 8.2
12–19........	38.1	56.0	−17.9	13.2	6.0	+ 7.2
19–26........	30.0	40.9	−10.9	17.8	2.0	+15.8
26–Ap. 2.....	21.6	44.4	−22.8	8.5	1.5	+ 7.0

system. In the course of the week period, from March 19th to 26th, for the space of 48 hours the amount of sodium choride in the food was considerably increased in hopes of producing an increased elimination of chlorine, and with it the products of metabolism and suboxidation to which toxic effects at that time observed in the patient might be attributed, but the only result obtained by this treatment at so late a stage was an increase in the œdema of the patient. The total of the eight weeks shows a net loss of 77.5 grams of nitrogen and a gain of 46.1 grams of sodium chloride. There are, of course, in such work innumerable sources of experimental error due principally to negligence on the part of those whose duty it is to keep an exact record of the amount actually consumed by the patient in the course of 24 hours, but having due regard for the influence exerted by all such possible causes of error there can be no doubt that whilst a heavy loss of nitrogen was suffered by the system during the last six weeks, a correspondingly large retention of chlorine must have taken place. The loss of 77.5 grams nitrogen would correspond with a loss in weight of 2¼ kilo on the part of the patient whilst the retention of 46.1 grams of sodium chloride, if present in the form of œdematous fluid throughout the body, should involve a retention of a very large amount of water, at least four to six liters, and to about 40 grams of nitrogen which would otherwise have been eliminated. A certain proportion of the chlorides in question undoubtedly were present in such fluids, for especially during the last 25 days a very marked œdema of the extremities was to be observed, and at the autopsy large quantities of fluid were found in the peritoneal and pleural cavities as well as a very considerably enlarged liver and spleen, but it is scarcely possible that the retention of such a large amount of chlorine salts can be explained on the above basis, and it is highly probable that the presence of suboxidation nitrogenous products capable of forming double compounds with sodium chloride and thereby materially interfering with its dissociation in solution may account for a considerable portion of the discrepancy. It is also probable from analyses made on the tumor and other tissues that an increase of chlorides had taken place not only in the blood and body fluids but also in the tissue cells, more especially those involved in the tumor. Analyses of blood serum taken from the heart within six hours of the death of the patient and of fluids of the brain, the pleural and peritoneal cavities showed on analyses a chlorine content varying from .38 per cent to .4 per cent; the freezing points from .61 to .64° C., both of which quantities are considerably in excess of the normal.

2. *The Influence of Variations in the Quantities of Chlorides in the Diet.*

An inspection of the figures Table II will show that the patient was practically in a state of chlorine starvation, the daily average amount being little in excess of a gram of chlorine, or 1⅔ grams sodium chloride. The experiment in the course of the week, March 19-26, during which the amount of chlorides in the diet was increased to the extent of several grams, has already been referred to. During this period the chlorides to the extent of 15.8 grams were retained in the system, and in spite of a loss of nitrogen an increase in body weight of a kilo and a quarter, accompanied by a great increase in œdema, was to be observed. It is interesting to note that during this period the nitrogen loss was less than in either the preceding or following weeks, a fact which may presumably be attributed to the retention of nitrogenous waste products in the œdematous fluids. On once more reducing the chlorides to a figure lower than in any previous week the increase in œdema was no longer as marked, but the death of the patient at the end of the week in question brought the series of experiments to a close.

3. *The Relative Proportions of Nitrogenous Constituents, etc., in the Urine.*

Since this question will be dealt with in detail in a subsequent portion of the report dealing with the diagnosis of cancer by means of the urinalyses it will not be necessary to give any

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[NOTE.—The secretion of the skin should not be entirely neglected, as the proportion of chlorides eliminated in that manner is much larger than is that of other constituents in comparison with the urine. See p. 100].

figures at this stage. A table has been prepared showing the relationship of uric acid, ammonia, chlorine and phosphorus to the total nitrogen in weekly periods. A steady gradual rise in the proportion of uric acid nitrogen to total nitrogen, a considerable fall in proportion of chlorine to nitrogen with a fairly steady ammonia and phosphorus proportion is to be noted.

CASE III.

Mr. U.; age 60; weight 75 kilo.; German; married; occupation brewer.—No family history of cancer. Personal history good. Consumed much beer but was not intemperate. About one year ago patient began to complain of vague pain, high up in the epigastric region, which continued with some remissions and became more and more troublesome. Very soon distinct symptoms of stenosis of the œsophagus developed, such as pain in the chest on swallowing, increasing difficulty in getting solid food through and later regurgitation of food immediately after swallowing. The pain also increased and became more continuous, and six months later definite diagnosis of carinomatous stenosis of the œsophagus a few inches above the stomach was made by his physician. Difficulty in swallowing even soft foods became more marked until finally only liquids could be passed through with difficulty. Frequent attempts at dilatation of the stricture were unsuccessful. Location of the cancerous involvement was made out definitely by œsophageal sounds. August, 1903, the occlusion was complete, so that not even liquid foods passed into the stomach and starvation was imminent. Patient suffered more from the extreme thirst than from hunger, and insisted on an operation which would give him at least relief from these symptoms. A gastrostomy was then performed and the operator was unable to discover any disease of the stomach. At the time of this examination patient has for over two months been fed through the fistulous opening. On inspection he shows no emaciation or marked cachexia. He has great difficulty in breathing and coughs violently at every attempt at speaking. This feature of his illness he complains of most. He is quite helpless and feeble, complains of headache and confusion due in part to constipation and in part to the regular use of morphia which is prescribed for the relief of pain. Physical examination shows a fairly well nourished body. Ascultation of the chest reveals no great abnormalities of the heart, but it is somewhat weak and rapid and irregular beat. In the lungs the larger bronchi are full of secretions on both sides. Rales, wheezing and shallow breathing in both; signs of pleurisy, or solidification of lung substance. Palpation of the abdomen reveals an enlarged liver pushing far over the stomach with no distinguishable signs of tumor but giving much pain on pressure, especially in the epigastric region. There is a considerable cushion of abdominal fat which makes accurate diagnosis difficult.

Course of the disease.—Patient was under observation five weeks. As stated above, he was, when first seen, dull, emotional and markedly toxic due at least in part to overfeeding of eggs and meat and a constipated condition with much flatus favored by the regular use of morphia to produce sleep and to relieve obstinate cough. A brisk cathartic had been given by his physician, and at our suggestion the amount of meat and number of eggs fed during the twenty-four hours was reduced, while more vegetable food was added so that the total nitrogen content of the meals averaged 9.5. A small dose of Carlsbad salt was prescribed to be taken every morning for a week, and his stomach was washed out four hours after each meal. When test meals were given the stomach was again washed out immediately before feeding and the food allowed to remain an hour and a half to two hours. This change was followed by marked improvement so that less sedative was required; sleep became more normal and the bowels more regular; there was less flatus and less cough. A marked improvement was also noticeable in the general appearance and conduct of the patient. He increased in weight, his color was better, he was clearer in his head, was less emotional and irritable and much stronger. At the end of two weeks he sat up most of the day, was bright and active mentally, read part of the time and was able to walk about and go out of doors on good days. This condition continued until the last week of patient's life, when increasing difficulty in breathing again became prominent, with much cough and need for sedatives and expectorants. The bowels became troublesome, toxic symptoms supervened, the heart became weaker and pulse more rapid. At the same time cachexia became marked with rapid emaciation and increasing weakness. He soon fell into a semicomatose condition and died on November 17, 1903.

Death occurred November 17, 1903.—Autopsy the following morning revealed the following conditions: The skin about the fistula was puffy and full of granulations, but with no appearance of cancerous involvement. On opening the chest the lung tissue was found normal, but there were slight pleuritic adhesions at the base of both lungs. Nothing unusual about the heart. Bronchial glands slightly enlarged and pulpy. Three inches of the œsophagus, immediately above the diaphragm, consisted of a large tumor mass completely occluding the lumen of the tube. The mass was about an inch and three-quarters in thickness and when opened was full of necrotic tissue. The cardiac end of the stomach was normal. The pylorus and the entire upper portion of the stomach showed nodules of various sizes beneath the mucosa, but no ulceration. The pancreas was normal. A few of the mesenteric glands were slightly enlarged. The liver was large, extending below the ribs and a considerable distance over the pyloric end of the stomach and contained numerous large foci of cancer varying from the size of a pigeon's egg to that of a pea. Other abdominal organs were normal.

For a period of six days exact analyses were carried out on the food and excreta with a view to determining the point of nitrogen and chlorine equilibrium, also the influence of an excess of chlorides administered in conjunction with the diet. The patient received three meals a day in the form of a semiliquid vegetable soup to which a small proportion of meat had been added. The principal object of this series of experiments was to observe the effect produced upon the general condition of the patient, the excretion of urine, more especially of nitrogen, ammonia, uric acid and phosphates, employing the diet referred to above as being most favorable, but for one period reducing the consumption of salt (NaCl) to a minimum, and in a second period increasing the amount of salt taken with the food to a quantity far in excess of the normal.

The accompanying Table III gives a comparison of the nitrogen and chlorine of the diet with the nitrogen, chlorine and uric acid of the secretions.

TABLE III.

DATE.	Nitrogen of food.	Nitrogen of urine and fæces.	Chlorine of food as *NaCl*.	Chlorine of urine and fæces as *NaCl*.	Uric acid.
Oct. 20–21	9.58	8.11	.98	3.88	.46
21–22	9.02	9.20	1.73	3.25	.45
22–23	9.04	7.63	1.88	1.49	.35
24–25	9.74	9.28	8.20	3.43	.38
25–26	9.73	8.50	9.89	7.96	.70
26–27	9.43	8.10	9.95	6.70	.52

The total volume of urine excreted in the first three-day period of small chlorine consumption was found to be extremely large, being about six liters. In the second period, when large quantities of salt were present in the diet, the total volume of urine was less than four liters, pointing towards a probable retention of fluid in the tissue, which was indicated to a certain extent by a slight increase in the œdematous condition of the patient.

The total nitrogen secreted in the urine showed practically no difference for the two periods. In the first 22.8 grams were eliminated and in the second 22.75; thus showing that the reduced volume of urine had not materially affected the removal of nitrogenous products. To the nitrogen secreted with the urine must be added that which was found in the fæces, amounting in the first period to 2.1 and in the second to 3.1 grams, giving totals of 24.85 and 25.9 respectively, which, when deducted from the nitrogen of the diet, 27.75 and 28.9 respectively, give differences of 2.9 and 3 grams, or an average retention by the system of one gram of nitrogen per day, which must be attributed to the improved state of the patient and possibly to a certain extent to the increase in œdema; but it is interesting to note that the amounts retained are exactly the same for the two periods in spite of the much larger retention of water and salt in the latter period.

Whilst in the first three-day period the total sodium chloride consumed with the food was only 4.6 grams, the sodium chloride secreted in the urine was equal to 8.24 grams and that of the fæces to .4 grams; in the succeeding three-day period, when the chlorine of the food equaled 28 grams, the amount secreted in the urine was only 17.64 grams and that in the fæces .45 grams, showing a marked tendency on the part of the system to maintain a chlorine equilibrium more or less following the laws of osmosis between the chlorides of the system dissolved in the blood, tissues, etc., and those of the kidney secretions. An examination of the amounts lost from day to day emphasizes this point, a fall to a minimum being observed at the end of the starvation period and a rise to a maximum in the course of the following three days of chlorine excess. But it should be observed that the total amount eliminated from the system in this period of time was only 26.7 grams, whilst 32.6 were introduced with the food. This tendency to retain a portion of the chlorine, taken in conjunction with the less marked retention of nitrogen referred to in the last section, may possibly be attributed partially to the increase in cancer tissue and to œdema, but also probably to a considerable extent to its association with products of suboxidation retained in the system.

The ammonia secretion in the first period was very large, reaching a total of 4.1 grams as against 1.75 in the second period, showing a marked improvement in the presence of large quantities of chloride.

The removal of uric acid and other bodies capable of reducing permanganate was, on the other hand, found to be larger in the second period than during the first. Whilst in the first period, with a limited chlorine diet and in spite of the large volume of the urine, only 1.26 grams of uric acid were removed, in the second period 1.6 grams were excreted with the urine.

Phosphates, which were absolutely constant in the diet, were also found to be practically constant in the urine and fæces, showing an average from 2 to 2.2 grams H_3PO_4 per diem.

An analysis of the blood showed a freezing point .57° and sodium chloride content .47 per cent, both of which are practically normal.

To summarize the results in this case it may be said that in the absence of toxic symptoms a practical equilibrium of nitrogen is established and a small tendency to retain chlorides exhibited.

CASE IV.

Mr. T.; age 66; Scotch; married.—Patient gives no family history of cancer or of tuberculosis. He has not had specific disease but has been intemperate. He gives an indefinite history of ten years' illness, referred particularly to the abdomen and kidneys. For the last few months he has had frequent urination, and the specimen contained varying quantities of blood. In the past year he has lost weight progressively and for the past five months has been confined to bed.

Patient died January 17, 1903, at the Buffalo General Hospital, and autopsy revealed the following conditions: Very marked emaciation. On opening the chest the right pleural cavity showed several large tumors the size of a plum, with cancerous disease of several ribs on the same side. The lung tissue was quite normal, and the bronchial glands were not diseased. In the abdominal cavity the lymph glands beneath the peritoneum were enlarged and hard. The stomach was free from cancerous change. The liver showed in the upper portion of the right lobe a slight metastatic carcinomatous growth. The mesenteric glands were not noticeably changed. The left kidney was large and soft while the right kidney was the seat of a large primary, carcinomatous tumor.

In this case a balance of the nitrogen and chlorine of the food and excreta was carried out for the nine days immediately preceding the death of the patient (see Table IV). The diet consisted exclusively of one or two eggnogs daily, the exact nitrogen and chlorine content of which was known. For two days a slight increase in the salt of the food was not associated with any considerable increase in that of the urine, but patient showed more marked œdema and became extremely cachectic. An examination of the table will show that whilst a steady loss of nitrogen was exhibited for the entire period, there was a strong tendency to retain chlorides in the system, and, as stated above, a slight increase in the chlorides of the food did not lead to greatly increased excretions and simply tended to accentuate the œdema of the patient. The uric acid nitrogen in comparison with the total nitrogen of the urine was high, varying from 1.7 to 2 per cent. The ammonia was above the normal from 5 to 8 per cent. In this case, in which the functions of the kidneys were materially interfered with, we have a marked illustration of the toxic symptoms associated with a considerable loss of nitrogen and a tendency to retain chlorides.

TABLE IV.

	Nitrogen of food.	Nitrogen of excreta.	Chlorine of food as *Na Cl.*	Chlorine of excreta as *Na Cl.*
8–9	5.1	8.3	2.0	.32
9–10	5.1	8.6	2.0	.32
10–11	5.1	10.5	2.0	.62
11–12	2.6	3.9	1.0	.08
12–13	5.1	5.4	2.0	.33
13–14	5.1	6.3	3.6	.55
14–15	5.1	6.8	3.6	.82
15–16	5.1	7.1	2.0	.45
16–17	2.6	4.5	1.0	.10
Total	40.9	61.4	19.2	3.59

CASE V.

Mr. C.; age 75; weight 65 kilo.—Sarcoma of skin and connective tissue. Patient presents a nodular discolored area in right epigastric region having a maximum diameter of six inches. Numerous tumor nodules, some of them as large as a walnut, apparently seated in the skin and the subcutaneous tissue, in the discolored area. Patient refused to submit to operation and left the hospital at the completion of the series of experiments. Whilst exhibiting toxic symptoms, his appetite was normal and nutrition apparently unaffected. For the twelve days that he remained in the hospital he received a diet containing approximately 11½ grams nitrogen and 8 grams sodium chloride per diem.

The analyses of urine and fæces were conducted with as great accuracy as those previously referred to, but in the diet a possible source of error is introduced, since analyses were only carried out on two days in the course of the experiment, giving 11.3 and 11.6 grams nitrogen and 8 and 8.1 grams sodium chloride respectively; on the remaining days the food being weighed to correspond as nearly as possible to the same amount. Our principal reason for including this case is the evidence which it affords of both a nitrogen and chlorine balance, even in the presence of a tumor which has reached considerable dimensions and in spite of marked toxic symptoms, provided the digestive organs are unaffected. A slight gain of nitrogen was apparently effected during each period, and the chlorides determined for the second six days showed a similar close relationship (see Table V); all of which corresponds with the body weight of the patient, which remained constant throughout the experiments. It is also interesting to note that in this case the uric acid nitrogen was averaging 1.4 per cent of the total nitrogen.

TABLE V.

Day.	Total nitrogen of food.	NITROGEN OF		Sodium chloride in food.	SODIUM CHLORIDE IN	
		Urine.	Fæces.		Urine.	Fæces.
1..	Approximately	9.4	for six days.	....	....	....
2..		7.3		....	....	....
3..		9.4		....	....	....
4..		10.7		....	....	....
5..		8.1		....	....	....
6..		10.1		....	....	....
	69.0 to 71.0	55.0	10.50			
		65.5				
7..	Approximately	10.1	for six days.	Approximately.	8.2	Only traces; disregarded.
8..		9.0			6.7	
9..		9.3			8.1	
10..		9.1			8.2	
11..		9.8			8.2	
12..		10.5			9.5	
	69.0 to 71.0	57.8	9.7	46.0 to 48.0	48.9	
		67.5				

The analysis of the blood showed 2.5 per cent nitrogen and .48 per cent NaCl.

To summarize results in this case it may be said that, in spite of the age of the patient and the development of the tumor, practically no effect has been exerted on his nutrition, which is fully up to the normal standard of a healthy individual of his age.

CASE VI.

Mrs. N.; age 58; married; weight 44 kilo.—No family history of cancer or tuberculosis. The patient can give no definite account of the duration of the disease except that it existed about a year, and that after it was first noticed in the vulva it was of considerable size and progressed very rapidly, particularly so in the past three months. On examination there is found extensive tumefaction of the right labium, nodular, hard and ulcerating on inner surface. There are also some nodules on the left side but not large. The entire vagina and cervix are involved in the tumor mass. There is much necrotic tissue and a dark, very offensive discharge. The growth on the right side is about the size of a man's fist. There are metastases in the immediate neighborhood of the right groin and perineum, also quite a large nodule in the right buttock. Patient is very much emaciated, feeble, cachectic and much of the time unable to take sufficient food, this last condition being aggravated by her mental state of depression. She has much pain, so much so that anodynes are required to give her rest and sleep.

In this case analyses were made on the 24-hour specimens of the urine for 45 days. For 24 days estimations of nitrogen and chlorine in the food, in the urine and the fæces were also carried out, but owing to the difficulty of properly controlling the patient only one period of six days can be looked upon as reliable.

1. *Nitrogen and chlorine equilibrium.*—For the first twelve days complete failure to effect an approximate balance of the nitrogen and chlorine led to the discovery that the patient was deliberately throwing away a portion of the urine and fæces, so that results were obtained similar to those of Moraczewski, showing a retention of nitrogen and chlorine, at the same time an appreciable loss in body weight being observed. Such results being obviously incorrect, for a period of six days patient was carefully watched and a fresh series of experiments showed a nitrogen consumption of 48.6 grams and a nitrogen loss of 54.2 grams.

At the same time 36.1 grams sodium chloride had been consumed with the diet and 34.2 grams excreted in the urine. Even these figures failed to afford a satisfactory balance, a loss of 5.6 grams nitrogen and a gain of 1.9 grams sodium chloride being scarcely comparable with the actual loss in body weight during this period of .75 kilo. One possible source of error, the secretions of the skin, will be considered at a later stage, but it must be concluded that cases of this type are not suitable for metabolism experiments, owing to the difficulty of controlling the loss which takes place continually from the surface of the ulcerating tumor. On the other hand, the 24-hour analyses may be made use of to compare the relative proportions of nitrogen, uric acid, ammonia, chlorine and phosphorus. The uric acid nitrogen was found as an average of the 45 days to be 2.1 per cent of the total nitrogen; the ammonia nitrogen 12.5 per cent; phosphorus 7.4 per cent and the chlorine 40 per cent of the total nitrogen.

*CASE VII.

P. G.; age 59; Polish.—Committed to Erie County Hospital March 14, 1904. Was found to be much emaciated; complained of inability to swallow solid foods but was able to take fluids. Examination with œsophageal bougies showed that there was some dilatation of the œsophagus and an obstruction at the cardiac opening. After several attempts, however, a moderately large bougie was passed into the stomach. He was kept under observation, and on May 21st a gastrostomy, typical Witzel, was performed. He was fed through the tube for about a fortnight, at which time the irritation in the œsophagus had so subsided that he was again able to swallow semisolid food with ease and some solid food. His condition steadily improved; he gained in weight and on June 15th tube was removed and shortly afterward the opening closed spontaneously. Patient's ability to swallow semisolid food and at times solid food remained unchanged until September, when he contracted lobar pneumonia, of which he died on October 5th.

The condition of the œsophagus on autopsy was shown to be slightly enlarged; there was no definite area of erosion or permanent stricture of the cardiac opening. The mucous membrane of the œsophagus was somewhat injected. The anatomical findings confirmed the clinical diagnosis of cardio-spasmus.

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*We are indebted to Dr. Gaylord of this Laboratory for above history and autopsy notes.

In view of the possibility that this was a case of cancer of the œsophagus a series of nitrogen and chloride determinations was carried out shortly after the operation had been performed. The patient's diet throughout this and subsequent experiments consisted of milk, with the addition of definite quantities of plantose (a vegetable albumen) and salt, meat broth and egg-nogs, which greatly facilitated the determinations of nitrogen and chlorine. The first series, *a*, as will be seen from the table, shows a distinct loss of nitrogen and fair equilibrium of chlorides. A heavy nitrogen loss at this stage must, however, be attributed to the shock of the operation and to the toxic effects of an infection which developed at the point at which the tube was introduced. A subsequent series, *b*, of nitrogen determinations carried out six weeks after the operation and previous to the development of pneumonia shows a very fair maintenance of nitrogen equilibrium. With regard to the chloride metabolism, it is interesting to note in series *a* that, whilst a heavy loss of nitrogen had taken place, probably owing to toxic influences, a very fair chloride equilibrium was maintained. There was no tendency to œdema, and the toxic influences referred to above apparently failed to exert such an effect upon the kidneys as to lead to a retention of sodium chloride.

TABLE VI.

Series a.

DAY.	Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
1	12.5	15.7	5.5	4.8
2	11.4	18.6	4.2	4.3
3	12.3	16.6	6.3	6.4
4	12.8	17.7	4.3	5.7
5	12.6	16.9	6.8	6.9

Series b.

DAY.	Nitrogen of food.	Nitrogen of excreta.
1	14.0	14.3
2	13.6	10.4
3	13.4	15.9
4	13.9	13.3

Estimations of uric acid and ammonia as compared with the total nitrogen of the urine over an extended period showed very low uric acid and fairly high ammonia results, the uric acid nitrogen averaging less than .8 per cent of the total nitrogen, the ammonia nitrogen, especially during the toxic period, being as high as 10 to 15 per cent of the total nitrogen.

*CASE VIII.

L. L.; age 44; weight 150 pounds; married.—Born in the United States of German parentage. Retired butcher. No family history of cancer or tuberculosis on either side. No personal history of syphilis or intemperance. Patient enjoyed ordinarily good health until the year 1897, when he states that he had an illness of two months duration characterized by much pain in the region of liver and marked jaundice, indigestion and fever. No evidence of gall stones. In April, 1904, had pain in the right hypochondria, but no vomiting, belching or other gastric symptoms. From July 1st experienced considerable difficulty in swallowing solids, and some with regard to liquids; belched gas and had small regurgitation of food with considerable mucus, but without noticeable color, odor or taste. At this time he suffered from sharp epigastric pain, which was almost continuous, and at the same time from constipation. A physical examination revealed nothing abnormal as to liver, stomach, heart, kidneys or appendix. On July 26th the œsophagus was sounded. The largest sized bulbs entered easily to a depth of 29 cm. from the incisors. Size 26 (French) entered to a depth of 30 to 31 cm., varying according to force employed but could not be inserted lower than 31 cm. Size 22 passed the stricture with some difficulty and it was thus determined that the lower œsophagus was sufficiently large to accommodate this bulb below a point 33 cm. from the incisors. On August 8th an X-ray examination was made, but revealed nothing as to the cause of stricture. On October 11th an attempt was made under ether to inspect the stricture by means of the speculum, but on account of a stoop of the shoulders it was not possible to straighten the spinal column sufficiently. At various times œsophageal bulbs were passed corroborating the results of the first examination and showing neither increase nor abatement of the stricture. No blood nor tissue was at any time found, the microscopical examination revealing nothing but epithelial scales and food remnants. Patient had lost weight considerably previous to the 26th of July, on which date he weighed 150 pounds. The experiments in metabolism referred to later were carried out between the first of August and the middle of October, at which time he weighed 158 pounds, having gained eight pounds in the course of the experiments. A careful investigation reveals no history of tuberculosis, syphilis or traumatism including corrosion, and since cardio-spasmus can be ruled out, reasoning by exclusion the case is probably one of cancer of the œsophagus.

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* We are indebted to Dr. A. L. Benedict of this city for the above history of the case.

From the above history it will be seen that, as the nature of the obstruction of the œsophagus is not known, it can not be said positively whether a tumor is present or not. A series of nitrogen and chlorine equilibrium experiments were carried out in conjunction with work on the nutrition of patient and the results over an extended period are included, so that they may be compared with those obtained on the cases known positively to be cancer, Nos. II, III, IV, V and VI, with the normal individual case (I) and the case of cardio-spasmus (VII). By the addition of plantose (a pure vegetable albumen) to milk, in varying proportions, it was found possible to convey sufficient food to the stomach of the patient to bring about a gain of 6 kilo in weight in the course of three months. The first series of experiments was conducted with a view to determine the amount of plantose which could most suitably be added to milk and eggnogs in order to give the requisite amount of proteid to maintain the nitrogen equilibrium between the food and excreta. For this period patient's diet consisted of an eggnog once daily, and 250 c.c. of milk with the addition of 20 grams of plantose and 3 grams of salt, three times daily, with milk in addition according to the desire of the patient.

From Table VII, in which the results of this first period are recorded, it will be seen that the loss of nitrogen, averaging 14.6, is only slightly in excess of the nitrogen of the food, averaging 14.4, quantities fairly in accord with the weight of the patient. During this period the chloride equilibrium was not determined with any accuracy, the results indicating a slight tendency to salt retention.

During the second period the nitrogen of the food was diminished to an average of 10.9 grams daily, with the result that a distinct loss of nitrogen was suffered by the system, averaging 1.7 grams daily. The total nitrogen secreted in the urine and fæces, averaging 12.6 grams, is not, however, indicative of toxic influences leading to abnormal destruction of body proteids, a fact which is further confirmed by a relatively low uric acid nitrogen. During this period the chlorides were maintained at a fairly high level, no very considerable differences being observed, an average of .9 grams sodium chloride being retained in the system daily.

TABLE VII.

Period 1.

Nitrogen of food.	Nitrogen of excreta.
13.0	14.6
13.0	13.1
14.2	15.2
15.5	14.5
15.5	14.4
15.5	16.2

Period 2.

Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
11.4	13.3	12.3	11.2
10.7	12.3	11.7	12.4
10.7	12.3	11.7	9.4

Period 3.

Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
16.8	14.8	11.7	9.5
16.8	17.6	11.7	11.9
16.9	15.6	11.7	10.9
17.0	13.6		
17.1	14.6		

In the subsequent Period 3 the amount of proteid in the food was materially increased to an average of 16.8 to 16.9 grams daily, with the result that a distinct retention of nitrogen was to be observed. For the first three days of this period the nitrogen intake exceeded the loss by 2.5 grams and for the two following days by as much as 5.9 grams, but it was subsequently found possible to diminish the nitrogen content of the food to 15.5 to 16 grams, at which figure patient gained weight and showed a marked improvement, conditions which are seldom associated with the development of a malignant tumor. For the first three days of this period the chlorides were excreted in a perfectly normal manner, as much as 11.7 grams in the diet being accompanied by a loss of 11.1 in the urine. A determination of the uric acid nitrogen showed a uniform content of .9 to 1.0 per cent of total nitrogen throughout the entire period, a figure which is below that generally obtained by ourselves and other investigators in cases suffering from a malignant tumor. This evidence of the absence of toxic influences would not, however, in itself justify us in deciding against the probability of the presence of a tumor.

At this stage reference must be made to a source of error which has been ignored by practically all those who have devoted attention to work on metabolism. The secretions of the skin, whilst possessed of a very small solid residue in normal healthy individuals, may reach considerable dimensions under pathological conditions which necessitate a performance by the skin of a portion of the kidney function. The meager analytical data bearing on this subject to be found in the literature would indicate a normal loss of .1 to .2 grams nitrogen and .2 to .3 grams sodium chloride per diem, but records are to be found of quantities as large as .5 grams nitrogen and 1 to 1.5 grams sodium chloride having been excreted, under pathological conditions, by the skin. Whilst the sources of experimental error in such work are so enormous as to preclude the possibility of obtaining strictly accurate figures, it is perfectly conceivable that even larger quantities than the above might be removed in the space of 24 hours. In any case it appears probable that the loss of sodium chloride by this means is from two to three times that of nitrogen, and consequently would afford a partial explanation of the fact observed in the majority of our own cases, as well as in those of most previous investigators, that the ratio of sodium chloride to nitrogen is lower in the excreta than in the food consumed. Assuming a minimum loss of .1 grams nitrogen and .2 grams sodium chloride and a maximum of .5 nitrogen and 1 gram sodium chloride, it will be seen at a glance that whilst the huge discrepancies recorded by Moraczewski of 8 or 9 grams difference between the chlorides of the food and those of the urine and fæces would not be materially affected, such a quantity would go far to supply the deficiency of chlorides in the urine observed in certain of our cases.

This source of error may well be advanced as an explanation of the slight discrepancies frequently observed in the balances of body weight, nitrogen and chlorine intake and output, and the osmotic tension of sodium chloride retained in the system, which should involve a retention of water and consequently an increase of body weight. For example, in Case II the observed loss of 77.5 grams nitrogen and a retention of 25 grams chlorine, or 41 grams sodium chloride in a period of eight weeks, would be changed to a loss of 80.7 grams nitrogen and a retention of only 18.7 grams sodium chloride, assuming that a daily loss of .2 grams nitrogen and .4 grams sodium chloride had been suffered by the system through the skin secretions. These figures would correspond far more closely with the variations in weight of the patient, which show from first to last a gain of about half a kilo, presumably attributable to œdema. Such an explanation would remove any necessity for assuming an extraordinarily increased concentration of sodium chloride or diminished dissociation of that substance into ions in the body fluids and cells, the only other conceivable explanation of the discrepancies observed. In other cases in which the daily retention of sodium chloride averages from .5 to 1 gram it will be seen that the loss of this substance by the skin may produce an appreciable difference; it must, however, be said that in those cases in which the salt consumption is small any great loss by way of the skin is improbable.

The blood.—In view of the evidence afforded by some of the above cases that in the last stages of cancer a heavy loss of nitrogen resulting from the destruction of body tissue may be counterbalanced in its effect upon the body weight by a retention of chlorides leading to the formation of œdema, it seemed a matter of considerable importance to carry out analyses on the blood and body fluids of such cases to observe deviations from the normal. Whenever sufficiently large quantities of blood were procurable, estimations were made of the nitrogen, chlorine, phosphorus, the lowering of the freezing point and titrations to the end points of a series of indicators. Blood counts were carried out from time to time, but only as required for clinical purposes, since the very thorough work of Kronkiewicz on this subject, showing variations in reds from 1,800,000 to 6,000,000 and whites from 4,000 to 20,000, rendered further work in this direction superfluous. It is very much to be regretted that, owing to the risk of endangering the life of the patient, we were able to obtain blood in sufficient quantities in only one or two cases in which patient exhibited marked cachectic symptoms, and consequently it was seldom possible to perform all the analyses referred to above in addition to the tests for hæmolysins, etc., referred to previously in this report. We have, however, a series of analyses on cases in which tumors had reached considerable proportions and discrepancies in the nitrogen and chlorine equilibrium had already been remarked, and the results can scarcely be said to deviate from the normal to the extent that one would be led to expect from the literature. The nitrogen, chlorine and phosphorus results varied considerably, showing a slight tendency to diminished nitrogen and increased chlorine (see Table VIII), but scarcely to the same extent as did those of Moraczewski. Titrations carried out on the serum after separation from the clot showed with one exception results very little below the normal in alkalinity. N/50 acid was employed, and from the tabulated results in which the amount of acid required to neutralize 1 c.c. of serum to given end points are recorded, a comparison may be drawn between our cases and those of Rigler. This investigator observed a fall in the alkalinity, both of the blood as a whole and of the serum, in a series of diseases, reaching an acute stage shortly before death, and in case of recovery once more rising to the normal. Using lakmoid as an indicator, he found 1.8 to 2.2 c.c. n/50 acid was required to titrate 1 c.c. of normal blood serum, whilst in acute diseased conditions this figure sometimes fell as low as 1.2 and even 1.1. Our records, whilst slightly below the normal, never reached these figures. (See Table IX.)

Engelmann, in a paper on the freezing point of the blood of cancer patients, finds variations from .56, the normal, to .65 in inoperable carcinoma ventriculi. *We have at present examined only six cases, five of which lie within the normal limits from .56 to .60, one being slightly over the latter figure. We are not therefore able to bring forward any results in support of the generally accepted view that the osmotic pressure of the blood serum of advanced cancer cases is necessarily above the normal, but are rather inclined to consider such variations as attributable to deficient heart and kidney function. It is, however, our intention to deal with the question of blood analysis more thoroughly at a later date, making use of a series of cancer animals. Since, however, gross analyses are of little value in dealing with the disturbances in osmotic equilibrium of cancer-cells, owing to the

TABLE VIII.

Analyses of Blood of Cancer Cases.

	Nitrogen.	Sodium chloride.	Phosphorus.
Cancer of the breast.........	2.13	.48	.03
Cancer of the stomach.......	1.84	.47	.08
Cancer of the liver..........	2.24	.45	.035
Cancer of the stomach.......	2.30	.49	.04

TABLE IX.

Blood Serum (Cancer Cases).

	Phenolphthalein.	Rosolic acid.	Lacmoid paper.
Cancer of liver.............	—.2	.7	1.71
Cancer of liver and stomach.	—.4	.8	1.8
Cancer of stomach..........	....	.58	1.66
Cancer of intestines.........	....	....	2.2

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*Analyses on materials obtained at autopsies (see p. 86) are, of course, excluded from these results.

great delicacy of such variations, we are devoting attention rather to the osmotic equilibrium of the individual cell and to the interchange of salt ions capable of passing through the cell walls in the hope of thereby throwing some light on the questions as to whether or no specific toxic influences exerted on the protoplasm of the cells are bringing about changes in their viscosity and permeability.

Whilst it is out of the question to summarize in any satisfactory manner the numerous isolated details brought out in studies in metabolism, and the average of a large number of results gives no idea whatever of the course actually taken in the establishment of any state of equilibrium, we have included a table showing the averages of the nitrogen and chlorides of the food and excreta for considerable periods of time in a series of cancer cases compared with the normal. Such a table is necessarily influenced to a certain extent by experiments carried out (in the course of the period, an average of which is presented) with a view to observing the effect of nitrogen or chlorine starvation or the administering of an excess of either of these substances. It will, however, enable us to recognize any extensive loss or gain of nitrogen or chlorine suffered by the system as the disease develops.

Case I, a normal individual, was submitted to partial proteid starvation and at the same time received widely varying quantities of sodium chloride. As might be expected a slight nitrogen loss was suffered by the body, whilst a marked tendency to the establishment of chloride equilibrium was exhibited.

In Cases II and IV, both carcinoma, in which cachexia had intervened, and the patients were suffering from partial proteid starvation and succumbed to the disease at the completion of the experiments, as well as in other cases records of which are incomplete, a marked loss of nitrogen and retention of chlorides was to be observed. At the same time it must be noted that the excretion of 6.7 grams nitrogen daily is very little above that of the normal individual in a state of starvation, and it does not indicate that the toxic effects of the tumor have led to an excessive proteid decomposition in the body.

Case III is remarkable when it is remembered that the patient's stomach and liver were riddled with carcinoma, and yet so slight an interference exerted upon the nutrition that a retention of nitrogen as well as sodium chloride in the course of our experiments was observed. One source of error unfortunately neglected was the loss of nitrogen and chlorine, which took place from the mouth and throat on expectorating after coughing and the employment of a gargle. It was our intention to repeat the experiments in question, but unfortunately the rapid decline in the state of the patient, followed very shortly by death, excluded the possibility of further investigation in this case.

Case V shows how little effect a rapidly growing sarcoma may exert upon the nutrition, and the nitrogen and chlorine equilibrium of a patient, a practical balance of these bodies being obtained for a space of twelve days.

Case VI, a markedly emaciated and cachectic case of epithelioma of the uterus, showed in the course of our experiments a loss of nitrogen and a retention of chlorides. The differences observed were very slight, a fact which may possibly be attributed to experimental errors resulting from the difficulty of controlling a patient of this type.

In Case VII (cardio-spasmus) as the result of an operation and subsequent infection, the loss of nitrogen in the first period is very considerable, whilst a fair equilibrium of chlorides is maintained. In the subsequent period, patient having recovered from the infection, the nitrogen balance is about normal.

In the last case recorded in the tables, suffering from stricture of the œsophagus, the cause of which is unknown, whilst a nitrogen equilibrium is established on a daily consumption of proteid containing 14 grams nitrogen, a smaller quantity in the diet leads to a loss on the part of the body and an increased quantity to retention of nitrogen. There is possibly a slight tendency to retain chlorides in this case.

We have in Table XI included some of the cases investigated by Müller and Braunstein, from which it will be seen that whilst Müller records a daily loss of nitrogen exceeding 5 or 6 grams in six cases out of eight, Braunstein, whilst obtaining a fair equilibrium in the majority of cases, records one in which as much as 10 grams nitrogen was lost daily through the urine and fæces in excess of that present in the diet. The nitrogen results obtained by the latter investigator correspond fairly well with those which we have obtained; but whilst we have noted a distinct retention of sodium chloride in all cachectic carcinoma cases, he finds a loss in four cases out of five.

Table X.

CASE.		Nitrogen of food.	Nitrogen of excreta.	Sodium chloride of food.	Sodium chloride of excreta.
I Normal..................	a	13.0	13.4	11.4	11.9
	b	11.6	13.2	13.1	13.5
II Carcinoma of stomach and pancreas................	a	6.5	6.5	1.85	1.8
	b	5.5	6.7	1.6	.9
	c	4.3	6.7	1.9	.45
III Carcinoma of œsophagus involving stomach and liver....................		9.4	8.5	5.4	4.45
IV Carcinoma of kidney..........		4.5	6.8	2.1	.4
V Sarcoma of skin and connective tissue...........		11.5	11.1	7.8	8.1
VI Epithelioma of uterus, etc....		8.1	9.0	6.0	5.7
VII Cardio-spasmus...........	a	12.3	17.1	5.4	5.6
	b	13.7	13.5		
VIII Œsophageal stricture (cause to be determined. See page 97)...........	a	14.4	14.7		
	b	10.7	12.6	11.9	11.0
	c	16.9	15.2	11.7	10.8

Table XI.

CASE.	Nitrogen of food.	Nitrogen of excreta.	Sodium chloride of food.	Sodium chloride of excreta.	
V Carcinoma ventriculi ..	3.9	11.4	......	......	Müller
VI Carcinoma ventriculi ..	3.45	12.0	.....	......	“
VII Carcinoma ventriculi ..	2.45	9.1	......	......	“
VIII Carcinoma pancreatis..	1.7	6.0	......	......	“
IX Carcinoma mammæ ...	9.1	10.4	......	......	“
I Carcinoma œsophagi ..	7.7	6.5	5.1	5.6	Braunstein
VI Carcinoma œsophagi ..	7.7	7.8	7.0	7.8	“
VIII Carcinoma œsophagi...	7.2	10.3	4.4	3.7	“
IX Carcinoma ventriculi ..	8.8	10.6	7.3	8.5	“
X Carcinoma œsophagi ..	4.2	14.9	.690	.794	“

Moraczewski's results are exactly the reverse of those obtained by Müller and Braunstein. In one case of carcinoma of the uterus he records a retention of 43 grams chlorine and 52 grams nitrogen, the patient merely gaining one kilo in weight. In a case of carcinoma of the ventricle he observed a chlorine retention of 82 grams with a slightly larger variation in weight. In Case VI, carcinoma of the ventricle, with one kilo increase in weight in the course of eight days, a retention of 75 grams nitrogen and 45 grams chlorine was observed. Such proportions are obviously impossible. An increase of merely one kilo in body weight could not conceivably be associated with a retention of quantities of chlorides and nitrogen as large as those recorded in the first and last examples. One must, however, conclude from his publications that he considers his cases afford evidence of a strong chlorine and nitrogen retention, whilst Müller is convinced that the loss of nitrogen is in the majority of cases extremely heavy. Braunstein finds in one case only out of ten any considerable loss of nitrogen, in the remaining cases a slight loss in body weight was observed. Our results differ considerably from either those of Müller or Moraczewski and are more in accord with those of Braunstein. We are inclined to believe that in all cachectic carcinoma cases a loss of nitrogen and a gain of chlorine may be looked for, especially where there is any tendency to œdema, but we have not in our experience met with any such huge differences in the intake and output either of nitrogen or chlorine as are to be found in the tables of either of the three investigators referred to above.

It must be confessed that none of our own cases affords an absolutely satisfactory balance of the various factors referred to in the introduction, change of body weight, loss and gain of nitrogen and chlorine, increase in weight of tumor, loss of tissues and production of œdema. It may, however, safely be said that when allowance has been made for a possible slight loss of nitrogen and chlorine by way of the skin and mouth, and for the other sources of experimental error, all of which tend in the same direction, viz: to an apparent diminution of the nitrogen and chlorine secretions, a fair balance is to be struck in all cases in the metabolic equilibrium. Whilst there is still a margin of chloride retention which would lead one to suppose that the excess chlorine held in the body can not be accounted for exclusively on the basis of increased fluid content, still the differences are not nearly as large as in Moraczewski's cases, and a slightly increased concentration of sodium chloride in the tissues associated with a decreased dissociation of that substance so as not unduly to disturb the osmotic equilibrium, might afford the necessary explanation. Unfortunately for this theory, we have not been able to obtain chemical evidence of any marked changes in this direction in the blood and tissues; but it must be remembered that existing methods of handling such a problem are of necessity crude and the changes to be investigated extremely delicate.

As regards the effect of increasing the quantity of chlorides in the diet and the effect produced by this means on the œdema of the patient, it must be said that whilst normal individuals show a tendency to establish a chloride equilibrium with very great readiness in the space of from 24 to 48 hours, being capable of consuming large quantities of chlorides above the normal without any marked changes in the blood and tissue, cachectic cancer cases, especially those in which the kidneys are deficient in their activity, are incapable of reacting even to a small increase in the amount of salt, marked œdema resulting from any such increase, which only disappears on once more reducing the amount present in the food. For details regarding the effect on the body weight and the secretion of metabolic products brought about by variations in the amount of chlorides in the diet, reference must be made to the analytical results recorded under each case.

The great significance of the role played by soluble chlorides in the processes of oxidation and elimination of waste products can not be overlooked, and we are not inclined to agree with Braunstein in assuming that the increase or decrease in the secretion of salt is simply a question of the amount of water retained in the system with which a certain amount of salt would normally be associated. We are inclined rather to look upon the water as being retained in conjunction with the salt, which in its turn is possibly prevented from proper diffusion by reduction in the permeability of membranes and by its possible association with products of metabolism. For the relative proportions of ammonia, uric acid and other nitrogenous constituents of the urine, phosphates, etc., reference must be made to the individual cases, or to a subsequent article in this report, in which this question is dealt with more in detail. Whilst increased secretions of uric acid and ammonia in proportion to the total nitrogen are frequently observed and assumed to indicate respectively disintegration of tissue rich in nuclein and faulty oxidation, it must be borne in mind that variations in the nature of the food consumed can bring about very marked changes in the proportions of these substances, as may be seen in Cases VII and VIII.

In conclusion it must be said that whilst work of this nature affords considerable evidence of the presence in the system of toxic bodies capable of seriously interfering with the nutrition of the patient, there is no positive evidence that we have to deal in cancer with toxins of a specific type, such as are invariably associated with the course of development of diseases caused by bacteria and other recognized parasites. The presence of nitrogenous suboxidation products resulting from some interference with the metabolism of the cells may well exert an accumulative effect, and as the disease progresses cause all the symptoms with which we are familiar in cancer cachexia.

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CHEMICAL EXAMINATION OF THE URINE AS A MEANS OF DIAGNOSING THE POSITION OF A TUMOR AND OBSERVING THE COURSE OF ITS DEVELOPMENT.

By G. H. A. Clowes, Ph. D.

A paper dealing with this subject and summarizing the results obtained by previous investigators was published by Blumenthal in the report of the Krebsforschung Komité, 1902. The conclusions arrived at by this author possess a considerable amount of value, especially in view of the difficulties that are associated with a thorough investigation of the urine. As the result of analyses carried out on a series of cancer cases we are in a position to confirm for the most part the results recorded in his paper, but in certain cases variations worthy of note have been observed. For the literature reference must be made to the original article. In a previous portion of this report the question of retention of chlorides in the system has been completely dealt with, and it is therefore only necessary to repeat the statement that in cases of advanced carcinoma, where accurate analyses have been carried out on the diet and urine, a considerable loss of nitrogen and retention of chlorides has been observed. It has been suggested by Schöpp that the chlorine apparently retained in the system is really removed from the surface of ulcerating tumors. This explanation was not, however, applicable in any of the cases under observation, with the exception of Mrs. H., in which it received due consideration. That the total chlorides in the urine are so considerably diminished as to be present in proportions as low as one per cent to one-half per cent of the total nitrogen, or even to entirely disappear, whilst the proportion of chlorine to nitrogen in the food is still as 1 to 3, is a matter of daily observation, but it must be borne in mind that the extremely low secretion of chlorides observed by early investigators might frequently be attributed to the diminished proportions of chlorides contained in a milk or fluid diet as compared with a solid diet. In the cases which have been under observation in this Laboratory, so long as the chlorides in the diet were maintained at a normal level of not less than 3 or 4 grams chlorine per diem, and so long as the individual was capable of assimilating solid food, the proportions of chlorine excreted in comparison with that of nitrogen seldom fell below 50 per cent, and it was only when the fatal termination of the disease was approaching that such figures as 2 per cent, 1 per cent and even less were recorded. In fact, the results correspond fairly closely with those observed by Müller, etc., on starving individuals. The explanation offered by this author that the organ proteids which are relatively poor in chlorides were undergoing decomposition in place of the circulating proteids needs further support before it can be accepted.

In six cases in which exact determinations of phosphoric acid in comparison with nitrogen were carried out for a considerable period the amounts of phosphoric in proportion to nitrogen were found to be 6.8, 6.8, 7.2, 7.5, 7.7 and 7.8 per cent, giving an average of 7.3, which corresponds fairly closely with the highest results, 7.3, obtained by Müller, and is slightly in excess of the normal. As regards various nitrogen compounds, we have already dealt with the question of the total nitrogen excreted in comparison with the amount assimilated, and have found that when due allowance is made for all possible sources of waste the amount lost is usually slight and sometimes considerably in excess of that present in the food. The proportions of the various constituents differ widely. Whilst normal urine may be said to contain from 90 to 95 per cent of its nitrogen as urea, about 1.0 to 1.5 per cent as uric acid, 1.5 to 4 per cent as ammonia and the residue as extractive and amido acid nitrogen, similar proportions have been found to obtain in the very early stages of development of those cases of cancer in which the tumor produced no direct effect upon the metabolism and did not interfere with the nutrition. In all cases in which a marked cachectic condition had set in and where the nutrition of a patient was seriously impaired, either by toxic effects or the mechanical development of the tumor, results such as the following were to be observed: Urea nitrogen from 85 to 90 per cent; ammonia nitrogen from 6 to 12 and even 15 per cent; uric acid nitrogen from 1.8 to 2 and even 3 per cent, as compared with .9, 1.0, to 1.3 in other cases. It can not be too strongly emphasized that such results were only obtained in those cases in which inanition, accompanied by the pathological destruction of proteids, had reached an advanced stage. Also it must be remembered that the nature of the diet has a great effect on the uric acid secretion.

Halpern, in fractionating the nitrogenous constituents of pathological urines, presents four cases of cancer, two of them being carcinoma of the stomach with metastases in the liver, one carcinoma in the region of the porta hepatis and the fourth a carcinoma cardiæ. In the first case, carcinoma of the stomach with metastases in the liver, a low urea accompanied by high purin body nitrogen (including uric acid) high ammonia and normal extractive nitrogen were recorded. In the remaining three cases the proportions of the various constituents were not far removed from the normal, the extractive nitrogen being, however, rather high but not in any sense comparable with the high results obtained by Töpfer, Setti and Gumlich for extractive nitrogen, reaching figures from 10 to 23 per cent. We have not in the course of our work met with any such great deviations from the normal as are recorded by these authors, and in one case, carcinoma of the stomach with metastases in the liver, in which no very serious interference with the nutrition was to be observed, practically normal results were recorded for ammonia and uric acid.*

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*Experiments on a series of cases fractionating carefully the nitrogenous constituents of the urine are being carried out in the Laboratory at the present time and will be reported upon when completed.

We have been unsuccessful in an attempt to confirm the observation of Griffiths, who claims to have isolated a specific alkaloid having the formula $C_8H_5NO_3$ from cancer urine by extracting with ether in urine previously evaporated in vacuo and treated with alkali. Our experiments resulted in the separation of kreatinine, traces of xanthine bases and other normal urinary constituents, but not of any compound which could be looked upon as specifically associated with cancer. The observation made by Bouchard that the urine of cachectic cancer patients is more toxic than the normal was confirmed, but this may well be attributed to the larger proportion of normal sub-oxidation products; and, as stated above, the search for any specific ptomaine has been unsuccessful. A large proportion of cancer cases appear to succumb rather to starvation than to any toxic effects exerted directly by the tumor in its development. Any interference with the metabolism of the patient naturally leads to the production of toxic products which induce cachectic symptoms, and, as Müller observed, the noticeable difference between the loss of nitrogen suffered by the normal starving individual and one dying of starvation as the result of interference of a tumor is a slightly more rapid destruction of body proteid and elimination of nitrogen in excess of food consumed per kilo of body weight in the latter case over the former.

The secretion of phenol and indican in the urine in cases of stomach and intestinal cancer frequently reaches from two to four times the normal, but we have seen one case in which a stomach carcinoma failed to lead to the production of any increase in the urine indican. In two cases in which the pancreas was involved we failed to observe any indications of sugar secretion, and are not therefore prepared to consider the absence of sugar in the urine as sufficient proof that the pancreas is not involved.*

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*Since the completion of this paper a publication has appeared by Cammidge on the presence of oxidation products of glycerine in the urine and the diagnosis of pancreatic disturbances and of cancer of the pancreas. We are at the present time testing all cases for this reaction, but have not a sufficient amount of material to justify any expression of opinion regarding the value of this reaction for diagnostic purposes.

Destruction of the tissues rich in nuclein, the kidneys, liver, spleen, pancreas, etc., usually leads as might be expected to a slight increase in the proportion of uric acid and phosphates as compared with nitrogen, seldom amounting, however, to an addition of more than .5 per cent to the uric acid nitrogen and .5 to 1 per cent to the phosphates, as compared to the total nitrogen of the urine. Albumin occurs as would be expected in all cases in which the kidneys are involved, and frequently in cases in which the tumor is breaking down, but we have not been able to confirm the observation of von Noorden that it is present in appreciable quantities in over 70 per cent of all cancer cases.

Lactic acid is found occasionally in the urine in cases in which the liver or uterus is involved. We found it continually in one case of cancer of the uterus, but on the other hand it must be stated that in two cases where large metastases were found in the liver at the autopsy no lactic acid had been separated from the urine at any stage of the investigations carried on whilst the patients were under our observation. We have observed the presence of beta-oxybutyric acid and aceton in the urine of patients in a comatose condition, just as is the case in diabetes, and it is interesting to note that in one case at least this phenomenon was associated with a considerable diminution in the apparent alkalinity of the blood. Ehrlich's diazo reaction is, according to Blumenthal, invariably associated with ulcerating tumors. We have obtained a diazo reaction in a very marked degree in two or three cases in which ulceration had set in, but not invariably.

Whilst it would be out of the question to attempt to establish any hard and fast rules whereby a diagnosis could be effected by a study of the urine, considering the limited state of our knowledge at the present moment, a careful consideration of the various factors suggested by Blumenthal and for the most part confirmed by ourselves, has made it possible on one or two occasions to determine the exact location of the tumor. It is, however, well to bear in mind that we have very little evidence of direct activity on the part of the tumor cells leading to any specific effect upon the nature and proportion of the products excreted in the urine, the changes observed being for the most part those which would be induced by some interference with nutrition and the normal processes of oxidation, and also such as would be induced by secondary bacterial infections. A very extensive series of experiments carried out with a variety of group color reagents and also a series of biological tests for specific products which might be definitely associated with tumor cells have so far been resultless in so far as the urine is concerned, but there appears to be no reason why a continuation of work in this direction might not lead to some means of effecting an early diagnosis of cancer.

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ON THE SECRETION OF THE STOMACH IN CANCER AS COMPARED WITH THE NORMAL.

By G. H. A. CLOWES, Ph.D. and E. W. JEFFCOTT, Ph.B.

As a part of the routine work of this Laboratory in the course of the last two years upwards of 200 stomach contents have been submitted to a detailed chemical analysis. Whilst our primary object has been the early recognition of gastric cancer, the materials at our disposal, including as they do some 30 pathologically diagnosed cancer cases, may be utilized to throw some light on the problems of impaired nutrition and disturbed chemical equilibrium associated with this disease.

In a previous paper, an abstract of which has been included at the end of this report, the application of volumetric methods to the analysis of peptic digests has been discussed in detail (see p. 161). The use of various indicators for the direct estimation of acids and akalies will not therefore be further referred to in this paper, except in so far as the normal deviates from the pathological, and also to make comparisons between the rapid volumetric methods usually employed in clinical laboratories, and the accurate but more tedious gravimetric methods which are only available in a well-equipped chemical laboratory.

An attempt has been made to select the most accurate analytical methods and by their application to a series of normal and pathological cases to throw light on the following problems:

1. With given test meals to determine the relationship, if any, existing between the amount of hydrochloric acid secreted and the total of nitrogenous products brought into solution through the action of pepsin on readily precipitated proteids.

2. On the fractionation of the above products of proteid hydrolysis by means of a series of precipitants and the relationship, if any,

existing between such fractions and the titration results. Also the investigation of the value of the phosphotungstic and tryptophan tests for diagnosis of cancer.

3. Regarding the direct secretion of sodium chloride, phosphates, etc., as well as hydrochloric acid, and the possible conversion of hydrochloric acid into sodium chloride through interaction with the sodium salts of organic acids.

4. On the osmotic pressure of the stomach contents at various stages of digestion; the possible role played by physico-chemical phenomena in the mechanism of secretion, and the extent to which toxins or other poisons acting on the cell protoplasm could interfere with the normal state of chemical equilibrium, resulting in an entire or partial suspension of the acid secreting function, or the reverse.

Analytical methods employed.—It seems advisable at this stage to give a brief outline of the analytical methods we have employed so that no confusion may arise:

Volumetric titrations whenever possible on unfiltered as well as filtered contents (see p. 122) were made as follows:

Phloroglucinvanillin drops to faint pink end point on hot porcelain plate.

Alizarin to first color change, to faint red; occasionally when two figures are given, to purple.

Phenolphthalein to first pale pink.

Dimethyl-amido-azo-benzol, lakmoid and Porrier's blue were also employed occasionally.

For details reference must be made to abstract. (See p. 161.)

Estimation of phosphates.—The method at present employed in estimating acid phosphates was also found to be extremely faulty. In order to obtain an estimate of the actual amount of phosphates present and of the effect which they produce upon the titration, use was made of the two following points: (1) that the salt NaH_2PO_4 is converted into Na_2HPO_4 between the alizarin and phenolphthalein end point; and (2) that the salt Na_2HPO_4 may be converted into Na_3PO_4 or its equivalent $Ba_3(PO_4)_2$ between the phenolphthalein end point when that indicator is used alone and in the presence of barium chloride and excess alkali. And since it was shown that two of the three hydrogen atoms of the phosphoric acid are neutralized previous to the phenolphthalein end point in titration and one atom previous to the alizarin end point, allowance may be made for the phosphates by deducting the mean of the result obtained above from the alizarin end point and twice that quantity from the phenolphthalein end point.

The working method employed in all cases to which reference is made in this article is as follows: Filtered stomach contents (25 c.c. if possible) evaporated with a few drops of weak alkali, ashed, extracted with weak acid, filtered, boiled to drive off CO_2, made alkaline and boiled with pure n/10 NaOH to drive off ammonia, made up to definite volume and titrated from phenolphthalein to alizarin and from phenolphthalein to the same indicator after boiling with an excess of alkali in the presence of barium chloride.*

It is needless to say that the assumption of Töpfer that the period of titration from dimethyl-amido-azo-benzol to alizarin is attributable principally to acid phosphates, is incorrect.†

Estimation of chlorides.—In order to obtain accurate data regarding the excretion of hydrochloric acid and chlorides, it was found necessary to evaporate and incinerate the filtered and unfiltered stomach contents in all cases; and also for comparison's sake, to follow the same proceeding with a test meal, the duplicate of that which had been given to the patient. The following estimations were made in all cases in which there was sufficient material at our disposal; a slight modification of the methods of Martius and Lüttke being employed:

a. Chlorine present in the form of neutral inorganic salts other than ammonia by evaporating 10 c.c. of stomach contents and ashing without the addition of alkali, extracting with boiling water and estimating the chlorine, either gravimetrically, as silver chloride in a previously weighed Gooch crucible packed with asbestos, washing with nitric acid, water, alcohol and ether respectively and drying at 100°; or, volumetrically, by adding to the aqueous extract of ash an excess of n/10 solution of silver nitrate and titrating the unused silver by means of a standard solution of potassium sulpho cyanid, using ferric sulphate as indicator.

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* Am. J. of Pharmacy, vol. 75, Feby. 1903.

†It must be said that the presence of any considerable quantities of phosphates in a test meal is likely to materially affect the titrations, analyses, etc., and is therefore to be avoided whenever possible.

b. The estimation of total chlorine present in the form of inorganic salts of the alkalies, as ammonium chloride and as free or combined hydrochloric acid by addition of excess of dilute sodium hydrate, evaporation, ignition in a platinum crucible as in *a*, subsequent estimation of the chlorine as described above. An alternative gravimetric method which yielded almost identical results consisted in treating a definite quantity of the filtered stomach contents with fuming nitric acid in the presence of an excess of silver nitrate for some days until organic matter was destroyed, diluting with water and filtering through a Gooch crucible as described above. This method gives results almost equal to those obtained in a Carius tube and slightly higher than those obtained by the method previously described, possibly due to the fact that the evaporation and ignition of a carbonaceous residue in an open platinum vessel always leads to a slight loss of chlorides, even though the greatest precautions may be taken not to carry the process of ashing too far. Or a slight difference may be attributable to the inclusion of a very small quantity of organically combined chlorine which would be lost in all probability in the first process but absorbed by the silver nitrate in the second. In any case the loss is so slight that it may be ignored in the physiological chemical work in which the experimental error is necessarily higher than in that of a purely chemical nature.

c. In order to obtain an estimate of the influence attributable to ammonium, a body which seldom occurs in fresh stomach contents in any considerable quantity, an additional portion of 10 c.c. of the original stomach contents was neutralized exactly with n/10 alkali to the phenolphthalein end point, evaporated, ashed and treated as above.

It will thus be seen that by the above process of ashing stomach contents, extracting chlorides and estimating them either volumetrically or gravimetrically, we have obtained from *a*, chlorine present as inorganic salts (not including ammonia); from *c*, chlorine present as inorganic salts + chlorine present as hydrochloric acid free and combined; from *b*, chlorine present as inorganic salts, as hydrochloric acid free and combined and in the form of ammonium chloride. By subtracting *a* from *c* we have the total hydrochloric free and combined with proteids. By adding *a* to *b*—*c* we have the total chlorine present in the form of salts of the strong alkalies and ammonia.

d. An effort was also made to estimate the chlorine present as free hydrochloric acid by treating with barium carbonate, filtering and washing the residue and estimating the amount of barium which had passed into solution, the equivalent being assumed to be a measure of the actual free hydrochloric acid. Some of the results obtained by this method have been recorded but it is inaccurate, and since for reasons which will be referred to later it appears probable that the passage from absolutely free to entirely combined hydrochloric acid is not sharp but gradual and is a question entirely dependent upon the equilibrium of the chemical system concerned, we have not thought it worth while to continue this series of experiments.

Nitrogen.—Nitrogen was estimated in all cases in the filtered stomach contents, the same solution being employed as in the experiments enumerated above.

a. Duplicate quantities of 5 or 10 c.c. were employed for the estimation of total nitrogen by the Kjeldahl process, potassium sulphate being used to raise the temperature.

b. Ten c.c. rendered slightly acid to phloroglucinvanillin drops, if not already so, boiled and filtered and nitrogen estimated in filtrate.

c. Ten c.c. saturated with excess of zinc sulphate crystals, filtered and nitrogen estimated in filtrate.

d. Ten c.c. precipitated with tannin tartaric acid solution, recommended by Effront, filtered and nitrogen estimated in filtrate.

e. Ten to 20 c.c. of the filtered stomach contents were warmed, treated with an equal quantity of hot but not boiling solution containing 20 per cent phosphotungstic acid and 2 per cent sulphuric acid, shaken, allowed to settle and filtered, precipitate being washed with a 10 per cent phosphotungstic acid, nitrogen determined directly in the filtrate by means of the Kjeldahl method.

Estimation of lactic acid.—A qualitative test for lactic acid was first performed by evaporating 10 c.c. of the stomach contents with a small quantity of hydrochloric acid to half its bulk. The solution was then extracted in a separating funnel with 10 c.c. of pure ether. The etherial extract after separating was allowed to flow on to the top of a 1 per cent aqueous phenol solution to which a drop of ferric chloride had been added. At the points of contact of the two fluids a yellow rim was formed in the presence of lactic acid, affording a strong contrast to the purple of the neighboring liquid. A complete transformation into yellow would follow on shaking the mixture provided any appreciable quantities of lactic acid had originally been present. In case the presence of lactic acid was established by this preliminary test, the exact quantity was estimated directly by means of the usual iodine and thio method.

Filtration of stomach contents.—The question as to whether the best results may be obtained with or without filtration of stomach contents is one which is frequently raised in the literature. When an Ewald test meal is employed with a sufficient quantity of water (250 c.c.) no very considerable difference is made in the titration and chloride results, and since it is impossible to estimate the nitrogenous constituents without filtration, it seems advisable to use filtered rather than the original stomach contents, especially in view of the fact that in the clear solution the end points of indicators are rather sharper. On the other hand, we have frequently encountered cases in which for some reason or other an Ewald breakfast was unsatisfactory, as, for example, when a gastrostomy had been performed and the patient was fed through a tube. When a soup meal consisting principally of vegetable proteids is employed, a very material difference is to be observed between the filtered and unfiltered stomach contents. Whilst the osmotic pressure as estimated by the freezing point remains practically constant, not varying by more than .02° C. before and after filtration and the total quantity of chlorine present in the form of acid + salts, estimated by gravimetric methods, is also fairly constant, the titration results show a great difference as do the figures indicating the proportion of salts to hydrochloric acid. In other words, as will be seen by an inspection of Table XII, a much larger proportion of sodium chloride passes through the filter than remains behind, whilst a large proportion of the hydrochloric acid is retained by the insoluble or partially insoluble proteids left on the filter. In one case, for example, whilst the total chlorine was 7.3 before and 7.5 after filtering, the chlorine present as salts was 2.1 before and 4.0 after filtration, giving differences of 5.2 and 3.5 for the available hydrochloric acid in the solution. These figures correspond with the phenolphthalein end points, which show 7.0 before and 5.1 after filtration, the difference being attributable in both cases to the influence of phosphates and organic acids, the freezing point meanwhile remaining practically constant, only changing from .39° to .40° C. Now, since such differences occur very frequently and reach even larger proportions, it must be borne in mind that filtered stomach contents never actually permit

TABLE XII.

	F P	Phenolpht. end point	Total Cl	Salts Cl	HCl by Diff.	Phosphates
Case						
I Before filtration.	—.39°	7.0	7.3	2.1	5.2	1.6
I After filtration..	—.40°	5.1	7.5	4.0	3.5	1.4
II Before filtration.	—.38°	7.5	6.9	1.8	5.1	1.5
II After filtration..	—.39°	5.9	7.3	3.0	4.3	1.4

of an estimate of the total hydrochloric acid available for digestive purposes, and that when regular test meals containing large quantities of animal or vegetable proteids are administered, the error introduced by filtration may reach such considerable proportions as to entirely mask the presence of quite appreciable quantities of hydrochloric acid. More especially is this the case if, owing to weak pepsin, the proteids have not been reduced to a point at which they pass fairly readily through the meshes of an ordinary coarse filter paper. In one case the unfiltered stomach contents showed both volumetrically and gravimetrically 2.8 c.c. of hydrochloric acid free and combined with proteids, a quantity which, whilst below the normal, was very considerably in excess of that found in the filtrate, which was only 1 c.c. We have therefore made a practice of determining chlorides, phosphates, titrations, etc., in unfiltered contents, and also in filtered when material permitted. Total nitrogen and its various fractions could only be determined in filtered contents, and consequently all figures used in comparison with nitrogen were estimated in the same solution.

1. *Relationship between hydrochloric acid secretion and peptic activity.*—A series of preliminary experiments with artificial peptic digests using varying quantities of hydrochloric acid has shown us that after allowing for soluble nitrogenous products brought into solution by the acid alone the degree of peptic activity is fairly proportionate to the nitrogen present either in the clear centrifuged solution or in the filtrate from which the insoluble residues have been removed, provided a sufficient dilution is maintained and all the working conditions are constant and the period of activity limited to three or at most four hours. We have therefore felt justified in considering the total nitrogen present in filtered stomach contents (after allowing for the soluble nitrogenous bodies present in the original test meal) as proportional to the peptic activity. The determination of this total nitrogen as against the available free and combined hydrochloric acid in some 200 normal and pathological cases (showing equivalents from 1:1 to 10:1, the large majority lying between 2:1 and 3:1) has convinced us of the correctness of the view held by Pawlow that no constant relationship obtains between the peptic activity and the hydrochloric acid secretion. The addition of further quantities of hydrochloric acid to pathological contents which showed an absence of free acid frequently led to a greatly increased peptic activity as estimated by the nitrogen brought into solution; a further proof that peptic activity and hydrochloric secretion do not bear any direct relationship to one another.

A method suggested by Benedict for estimating the degree of peptic activity by precipitating the filtered stomach contents with an excess of phosphotungstic acid and centrifuging has not been found to give satisfactory results on comparison with the nitrogen content of the same filtrates.

2. *Fractionation of products of proteid hydrolysis; phosphotungstic and trytophan tests.*—The digestion of proteids in a normal stomach by means of pepsin hydrochloric was supposed until recently to stop

short at the formation of albumoses and peptones. Boas, Schaeffer, Hammerston, Gamgee, Neumeister, Foster, Beilstein and Hughes fail to make any reference in their works to the formation of bodies representing further hydrolysis of the peptones until the latter substances are submitted to the influence of tryptic digestion. Effront recognizes the presence of proteids not precipitated by phosphotungstic acid. Zunz refers to substances which fail to give the biuret reaction with copper; also in a subsequent paper to nitrogenous bodies giving a biuret reaction which are precipitated by phosphotungstic and not by picric acid. Pfaundler in dealing with the end products of peptic activity concludes that after precipitating albumoses with zinc sulphate and peptones with phosphotungstic acid, a residue of nitrogenous products remains in the filtrate which fails to give the biuret reaction which, on hydrolysis with strong acids, may be split up into amido bases and amido acids, the bases thus formed being readily precipitated by means of phosphotungstic acid.

Lawrow and Langstein established the existence of crystalline end products of peptic digestion. It must, however, be borne in mind that such bodies were only obtained by them in vitro after long periods of incubation.

Reach in a paper on the digestive processes in the stomach, referring to the work of Zunz, who found 90 per cent of the non-coagulable nitrogenous products present in the stomach contents of dogs in the form of albumoses, started an investigation as to whether the limit of further digestion were 10 per cent or whether the residual bodies formed were immediately absorbed owing to their lower molecular weight. He carried on his experiments with dogs, and fractionating the contents after four hours he found from 30 to 56 per cent of albumoses, from 35 to 20 per cent of peptones and from 31 to 32 per cent of end products, according to the quantity of proteid in the original test meal. The albumoses were precipitated by saturating with zinc sulphate and the peptones by the addition of picric acid to the filtrate from the albumoses.

Emerson, working with Müller, investigated the products obtained by fractional precipitation of the stomach contents of normal individuals and arrived at the conclusion that saturation with zinc sulphate gave a precipitate of albumoses containing 51.5 per cent of the total nitrogen; that further precipitation with warm concentrated phosphotungstic acid gave an additional precipitate of peptones containing 31.4 per cent of the total nitrogen, and that the residual filtrate, amounting to about 17 per cent, representing a more advanced stage of proteolysis, was fairly constant. In the stomach contents of a cancer case he found figures differing very considerably from the above, the albumose fraction being 25 per cent, peptone 47 per cent and the phosphotungstic filtrate over 27 per cent. He claimed that not only were such results to be obtained from the actual stomach contents but that the cancer itself must contain an enzyme capable of carrying peptic digestion to a more advanced stage. Equal weights of cancer tissue, one without heating and the other heated to 80° C. were added to two fibrin hydrochloric acid pepsin digestion mixtures, with the result that after several days the albumose precipitate in the heated test was much larger and the phosphotungstic filtrate much smaller than in that which had not been heated. Toluol was made use of to retard the development of bacteria, but toluol is a comparatively weak disinfectant for a long digestion and apparently no filtration experiments through bougies were carried out, and even had this been the case, previous bacterial development in the tumor might have influenced the result.

We have wherever possible made use of Ewald breakfasts or plain proteid test meals in stomachs which had previously been washed out with distilled water. After removing and filtering the contents when the quantity was sufficient (in addition to the titrations and the determinations of chlorides, phosphates, etc., referred to in another section) the nitrogen precipitate by boiling was determined, the nitrogen precipitate on saturation with zinc sulphate, the nitrogenous bodies precipitated with phosphotungstic and those present in the phosphotungstic filtrate. In certain cases the precipitate with bromine and the precipitate by means of tannin-tartaric acid solution were also determined. The precipitate on boiling was usually from 3 to 5 per cent. That obtained by saturating with zinc sulphate under normal conditions from 40 to 55 per cent, a result which was frequently fairly in agreement with that obtained from the tannin-tartaric acid. The phosphotungstic acid precipitate was usually about 80 per cent, leaving the phosphotungstic acid filtrate containing between 17 and 20 per cent of the total nitrogen.

In the accompanying Table XIII the results of fractionating normal stomach contents according to the above scheme are recorded. Three cases are typical of many others and will suffice as an illustration of normal results. Column 1 gives in cubic centimeters of a $\frac{1}{10}$ N solution the total nitrogen in 100 c.c. of stomach contents. Column 2 the nitrogen content of the bodies which have been precipitated by boiling. Column 3 the nitrogen of the zinc sulphate precipitate obtained by saturating the solution with that salt and subsequently washing the precipitate with a saturated solution. Column 4 gives the nitrogen of the phosphotungstic acid precipitate obtained by treating 10 c.c. of the warm stomach contents with 10 c.c. of the 20 per cent phosphotungstic acid solution (containing 2 per cent of sulphuric acid) referred to above. Column 5 gives the results of a similar precipitation with phosphomolybdic acid and 6 the nitrogen content separately determined of the filtrate from the phosphotungstic acid precipitate. It will be seen that in the first two cases the portion precipitated by boiling is about 3½ per cent; by saturating with zinc sulphate over 50 per cent; by precipitating with phosphotungstic or phosphomolybdic acid over 80 per cent, the resulting filtrate being about 19 per cent. In the third case, whilst the precipitate on boiling with phosphotungstic acid was about the same as in the first case, the zinc sulphate filtrate was considerably smaller, being about 37 per cent. The titrations of the same materials in Case I showed for 10 c.c. 3.5 c.c. to phloroglucinvanillin drops end point;

TABLE XIII.

Normal case	1	2	3	4	5	6
I.........	147	5	79	119	120	27
II.........	160	6	85	129	127	30
III.........	144	5	53	117	118	26

4.4 c.c. to the alizarin end point and 7.1 c.c. to the phenolphthalein end point, the difference of 2.7 between the two latter corresponding very closely with the phosphotungstic filtrate, a point which will be referred to at a later stage. The end point 7.1 c.c. for phenolphthalein after deducting .6 c.c. attributable to phosphates corresponds fairly with 6.3 c.c. of available hydrochloric acid estimated gravimetrically. The hydrochloric acid present is consequently equivalent to rather less than a half of the total nitrogen, a relationship which very frequently obtains.

In Table XIV a series of the most widely deviating pathological cases has been selected and will suffice to prove that not only is the above relationship of normal cases by no means constant, but also that certain pathological cases do not necessarily deviate from the normal as might be supposed from the literature. Cases 1 to 5 were microscopically and clinically and subsequently by autopsy diagnosed as cancer of the stomach, and it will be seen at a glance that while three show a high percentage of phosphotungstic acid filtrate the remaining two are perfectly normal, being under 20 per cent. On the other hand, cases 6, 7 and 8, which are not cancerous, 6 and 7 being gastric ulcers and 8 a case of hyperchlorhydria, may be selected as illustrating an extreme deviation from the normal, phosphotungstic filtrates being 46, 24 and 65 per cent respectively. The appended figures showing the amount of hydrochloric acid physiologically available in the stomach are simply introduced to show that abnormalities may occur either in the complete absence or in the presence of an excess of free hydrochloric acid.

TABLE XIV.

	1	2	3	4	5	6	7	8
Total nitrogen	158	185	162	110	150	116	210	91
Nitrogen in zinc sulphate ppt	70	86	98	30	45	55	35	10
Nitrogen in phosphotungstic filtrate	25	47	17	36	37	53	47	60
Physiologically available hydrochloric acid	18	23	Nil	12	Nil	66	45	90

After determining the nitrogen of the phosphotungstic filtrate as against the total nitrogen in some 200 stomach contents after proteid or Ewald test meals, we are by no means inclined to agree with Emerson in assuming that a greatly increased phosphotungstic filtrate may be looked upon as indicative of cancer of the stomach. The largest recorded analyses of 60 and 70 per cent referred to above were obtained in cases of hyperacidity. On the other hand, figures in excess of the normal were obtained in certain cases in which hydrochloric was practically absent and the bacterial enzymes had evidently played an active part. Whilst normal stomach contents, generally speaking, show from 18 to 22 per cent after from two to three hours digestion, and cancer cases frequently show as much as 40 per cent, we have had six cases of cancer of the stomach confirmed subsequently by autopsies, which showed from 10 to 20 per cent phosphotungstic filtrate, and 2 cases of gastric ulcer have given results even in excess of the highest obtained in cancer. The same relationship is found to obtain in dealing with Ewald's tryptophan reaction, which will be referred to later.*

Whilst there does not appear to be any constant relationship between the available hydrochloric acid present in a gastric digest and the total quantity of nitrogen in the solution (which may be taken as a criterion of the peptic activity) other definite proportions occur frequently in the various nitrogenous fractions which can scarcely be overlooked. When digestion has been allowed to take its proper course in the presence of a normal proportion of pepsin and hydrochloric acid for at least two to two and a half hours, whilst the total range of titration varies within very wide limits, as was shown in a previous paper referred to above, that portion lying between the alizarin and phenolphthalein end points is, after allowing for phosphates, frequently about 18 to 20 per cent of the total nitrogen equivalent. The phosphotungstic filtrate is also, as stated above, frequently about 20 per cent of the total nitrogen; consequently a very close equivalence exists between the range from alizarin to phenolphthalein on titration and the phosphotungstic filtrate. (See Table XV.)

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*In considering the apparent tendency to advanced proteid decomposition in cancer as compared with the normal resulting in an increased phosphotungstic filtrate, it is interesting to note the result of some experiments carried out in this Laboratory in the winter of 1902, having in view the production of an anti-pepsin by means of rabbit serum. Von Dungern has obtained an anti-pepsin by making use of geese and failed to obtain any reaction from the ordinary laboratory animals. Our results agree entirely with his in the main, the sole difference being the tendency to an increased phosphotungstic filtrate in those cases of peptic digestion to which the immune serum had been added, in comparison with those containing normal rabbit serum. This would indicate either a stimulus of the secondary digestive function of the stomach or a neutralization of some normal constituent, the function of which would be to restrain any tendency to the continuation of peptic digestion beyond a certain point. It is conceivable, although scarcely probable, that such a body controlling the digestive processes and protecting the cells from the action of peptic and other enzymes may be entirely destroyed in certain cancer cases. We are not, however, inclined to the belief that this is actually the case, considering it far more probable that the observed results are due to secondary antolytic or bacterial enzymes.

TABLE XV.

Case	1	2	3	4	5	6	7	8	9	10	Avg.
Alizarin to phenolphthalein	25	19	30	26	43	31	17	31	21	35	.....
Phosphates	4	2	5	4	5	4	1	5	3	5	.....
Difference	21	17	25	22	38	27	16	26	18	30	24.0
Total nitrogen	113	107	131	120	228	135	80	127	104	144	128.9
Phosphotungstic filtrate	23	19	23	27	40	26	18	24	19	27	24.6

In this table are recorded the titration differences between alizarin and phenolphthalein, the phosphates determined independently which should be deducted from the previous quantity in order to form a fair estimate of the influence attributable to some specific basic group combined with hydrochloric in the solution. The total nitrogen and the nitrogen of the phosphotungstic filtrate from 100 c.c. of stomach contents were also recorded in their n/10 equivalents. It will be seen at a glance that the closest relationship exists between the corrected titration and the phosphotungstic filtrate; further, that both these quantities are slightly less than one-fifth of the total nitrogen.

This relationship obtains in about 40 per cent of the pathological cases investigated, but is subject to wide and unaccountable variations which preclude its utilization as a means of diagnosis. It has been stated previously that where hydrochloric acid is deficient, or from some other cause stagnation or bacterial decomposition has occurred, the widest range of variation of titrations is encountered, and at the same time the phosphotungstic filtrate is likely to rise considerably above 20 per cent, its normal value. But the titration fraction from alizarin to phenolphthalein is not necessarily affected at the same time as the phosphotungstic filtrate, very frequently one remaining directly proportionate and the other being greatly increased or diminished, and that without any apparent rule, certain cases of well-marked cancer of the stomach exhibiting normal proportions and others deviation in one direction or the other, more generally, however, in the matter of the phosphotungstic filtrate.

Whilst the residual phosphotungstic filtrate shows a certain relationship with the titration results, it has not been found possible to trace out any connection between the so-called albumose fraction, precipitated by zinc sulphate, and the portion between zinc sulphate and phosphotungstic acid supposed to represent peptones and amido bases and the titration results. The period from drops to alizarin which probably represents some form of weakly combined hydrochloric fails to show, as might be expected, any proportionality with any of the above mentioned fractions of the nitrogenous constituents and can not therefore be made use of as a means of differentiating between the normal and pathological, except in those cases in which an abnormal bacterial disintegration of proteids resulting in the liberation of free acid groups shows titration results out of all comparison with the mineral acids actually present.

The observation made by Winternitz, Malfatti and Glaessner independently that tryptophan, one of the final products of tryptic digestion, is to be found amongst the products of peptic digestion, led to the examination of normal and diseased stomach contents for this substance as a possible means of diagnosing pathological conditions. Since tryptophan has been identified by Hopkins and Cole as an indol-amino propionic acid and is generally associated with leucin, tyrosin and similar products of final proteid digestion, and possesses in common with the latter the property of not being precipitated from a weak acid solution by means of phosphotungstic acid, its presence or absence might be expected to correspond with the increase or decrease in the size of the phosphotungstic filtrate. Glaessner tried the effect of feeding patients with proteid test meals, removing the contents and testing with bromine water in the presence of small quantities of acetic acid. He experimented with 36 cases, 12 of which were cancer, and obtained positive results in only 4 cases, all of which were highly acid and only one carcinoma. The same author tested the effect of adding stomach contents of cancer cases to a normal peptic digest, obtaining a positive result in only one case. On the other hand, he found that the addition of the actual tumor material to a normal pepsin-hydrochloric digestion led in the course of 24 hours to a strong tryptophan reaction with bromine, other tissues, normal stomach membrane, liver, etc., giving negative results. Erdmann and Winternitz in discussing the proteinochrom (tryptophan) occurring in the stomach contents, recommend the use of chlorine instead of bromine on account of the disturbing effect of an excess of the latter substance. They failed to obtain the reaction in any normal or pathological cases other than cancer, except where stagnation of the contents had led to secondary decomposition. In carcinoma they obtained a positive reaction by the addition of chlorine water, drop by drop, to filtered stomach contents in the large majority of cases, even in the absence of bacterial decomposition.

In the course of the last twelve months we have tested on suspected cancer cases both with chlorine and bromine in weak acid solution. Whilst stagnant stomach contents, especially in the presence of a large excess of acid, will frequently give a faint reaction, as will stomach contents in which bacteria have suffered no check owing to the absence of free hydrochloric, we have never succeeded in obtaining any marked reaction in cases in which the stomach had been carefully washed with distilled water before administering the test meal.

We have further attempted to follow out the method made use of by Emerson for the phosphotungstic filtrate and Glaessner and subsequently Erdmann and Winternitz in studying the trytophan reaction, of adding cancer of the stomach tissue immediately after the operation to artificial peptic digestion mixtures which were allowed to stand over night in the incubator. Even then the phosphotungstic filtrate was not necessarily greatly increased and the trytophan reaction was only obtained after incubation periods varying from 24 to 48 hours in some 40 per cent of the cancer cases. It was further observed that far the strongest reaction with chlorine was obtained when in the absence of disinfectants foreign organisms were allowed to develop whether the tissue employed were of a cancerous origin or not. We have not up to the present examined a sufficient number of cases to be justified in offering a positive opinion on the subject, but it can not be said that the evidence so far obtained would justify the assumption that the phosphotungstic and tryptophan reactions may be looked upon as indicating the presence in cancerous tissue of some peculiar enzyme capable of carrying proteid digestion to an abnormally advanced stage in the presence of free acids. The fact that the best reactions were invariably obtained in those cases in which advanced bacterial decomposition had already taken place inclines us to the belief that any such reaction is merely secondary and is not to be attributed to the direct influence of the carcinomatous cell and its contents. This is the more unfortunate since the existence of such a body capable of decomposing peptones with the formation of some of the final products of tryptic digestion might well explain the disturbances in equilibrium resulting in the suspension of hydrochloric acid secretion, etc., observed in cancer cases.

SECTION III. *Secretion of Chlorides, Hydrochloric Acid, Etc., in Cancer.*

Reissner in dealing with the estimation of hydrochloric acid in the stomach contents points out errors in practically all existing methods. He finally makes use of a modification of that of Martius and Lüttke. He decides after numerous experiments with free hydrochloric acid in the presence of chlorides, phosphates, peptones, organic acids, etc., to determine the physiological available hydrochloric acid by a method similar to that which we employ, the exception being that he titrated filtered stomach contents to the lakmus end point instead of phenolphthalein, and consequently found rather larger proportions of the chlorides present as ammonium salts. The method depends, as does that which we have employed, upon the assumption that in stomach contents, which are evaporated, charred and partially but not completely ashed, a point can be determined empirically at which the chlorides originally present will still be entirely retained and the hydrochloric acid which was present in the free state and combined with proteids or ammonia completely evaporated. Further, that by addition of alkali to a given end point one may include all the acid present in the free state and combined with weak bases present in the proteid molecule, without including that which was present in combination with ammonia; and that an excess of alkali will certainly include all chlorine originally present of whatsoever sort, provided too high temperatures are not employed in ashing. If the temperature is carried too far there can be no question that small quantities of chlorides are lost by direct vaporization; also that acid phosphates interacting with sodium chloride would eventually liberate hydrochloric acid. Reissner apparently attributes the low figures which he obtains partially to insufficient time permitted for the reaction to reach equilibrium after the addition of the exact amount of alkali required to neutralize the hydrochloric acid, or an excess. Also he suggests the possibility of neutral chlorides as calcium chloride interacting with acid potassium phosphate with the liberation of potassium chloride and hydrochloric acid, which is volatile. The actual state of equilibrium eventually established is entirely dependent upon the concentrations and the temperatures employed at various stages of the analysis. The simplest solution of such a problem is to avoid test meals containing any large quantities of phosphates, organic acids, ammonia, etc., and to make exact analyses of such test meals, a procedure which we have systematically adopted.

It has been noted in a previous section of this paper that the secretion of pepsin in the stomach is less readily influenced by the presence of a tumor than is that of free hydrochloric acid. Tests for the presence of free hydrochloric by means of phloroglucinvanillin drops, or of hydrochloric combined with proteids by the gravimetric methods referred to above may show absolutely negative results before the secreting cells have been seriously interfered with by the development of the tumor, a fact which excludes a purely histological explanation of the phenomena of impaired hydrochloric acid secretion. This extreme sensitiveness of the hydrochloric as compared with the pepsin secreting function of the cell is remarkable in view of the fact that mineral salts are chemically far less susceptible to changing conditions than are the enzymes and ferments. On the other hand, salts and mineral acids are electrolytes and consequently play a very important role in the establishment of osmotic equilibrium, and any disturbing influence in this direction may well be expected to affect them more easily than pepsin, a body which is presumably possessed of a large molecule and is incapable of playing any considerable role in osmosis.

Having observed in the course of our gravimetric work on the chlorides of the stomach contents that abnormally large quantities of sodium chloride are frequently found associated with very minute quantities of hydrochloric acid, the first point to be determined was to what extent sodium chloride might be secreted directly in the stomach in the place of free hydrochloric; also to what extent the hydrochloric when secreted might be directly neutralized by contact with the sodium salts of organic acids produced as products of suboxidation in the tumor itself. A long series of experiments was carried out on cancerous and non-cancerous cases as follows: Series of test meals were given containing definite quantities of sodium chloride, the greatest precaution being taken to remove all traces of chlorides from the stomach before commencing the experiments. After the usual period had been allowed to elapse the contents were removed, the residues washed out and the total quantity carefully analyzed. In practically all cases, both normal and abnormal, more sodium chloride was present in the stomach than had actually been introduced with the test meal. In normal cases this proportion seldom exceeded 5 to 10 per cent of the total amount of chlorine present, but in cancerous cases, especially those in which the tumor had already commenced to disintegrate, quantities of sodium chloride far in excess of those actually introduced and sometimes nearly equivalent to the normal proportion of hydrochloric acid were encountered. This was not, however, always the case, a total absence of hydrochloric acid being associated occasionally with an almost total absence of sodium chloride over and above the quantity actually introduced. Any neglect in the removal of the stagnant contents previous to administration of the test meal invariably showed a high proportion of sodium chloride, possibly owing to the increased volume of fluid affording an increased osmotic tension and increased secretion of salts and the presence of the sodium salts of organic acids which would react with any hydrochloric acid secreted. In one case, primary cancer of the oesophagus with involvement of the stomach and liver, a gastrostomy having been performed, the patient was fed entirely through a rubber tube. Such a case afforded an admirable opportunity of determining the effect exerted on the secretion of hydrochloric acid, sodium chloride and certain other mineral salts by introducing varying proportions of those substances directly into the stomach; the point being determined at which, owing to the high osmotic tension of the fluids introduced, no secretion was to be observed. It is not possible to employ an Ewald test meal in such a case, owing to the tendency of the bread, however finely divided, to block up the tube. A soup meal was therefore employed, determinations of nitrogen and chlorides being made in advance. The stomach was washed out with water twice before the administration of each meal. The meal was removed at the end of a period of an hour and a half and all residual material washed out with a fresh quantity of water. By determining the quantity of nitrogen present in the stomach contents it was possible to detect any loss which might have taken place through passage of the contents into the intestinal tract. Organic acids were not present in any large quantity, probably owing to the fact that all residues of meals left in the stomach after four hours were removed.

An inspection of Table XVI demonstrates fairly conclusively that sodium chloride, as such, must have been secreted directly by the cells of the stomach wall, especially in those cases in which no sodium chloride had been included in the diet. On the other hand, a large amount of sodium chloride in a test meal was usually associated with a loss of that substance in the stomach, and since the loss directly into the intestine was extremely small and could be allowed for, it must be supposed that chlorides actually found their way into the system by way of the stomach membrane.* The larger the quantity of hydrochloric introduced the smaller was the actual secretion of that substance and the larger the proportion of sodium chloride produced, indicating a possible reversing of the ordinary hydrochloric secretory functions, alkalies being in some way obtained

TABLE XVI.†

DATE	TEST MEAL			STOMACH CONTENTS		
	NaCl	HCl	Total	NaCl	HCl	Total
Oct. 8....	560	Nil	560	210	210	420
9....	500	"	500	225	150	375
10....	450	"	450	240	70	310
12....	315	"	315	137	140	277
13....	90	"	90	140	70	210
29....	420	"	420	237	87	324
31....	500	"	500	250	60	310
Nov. 3....	175	175	350	175	150	325
4....	280	200	480	230	170	400
5....	20	Nil	20	75	125	200
6....	10	180	190	90	160	250
7....	10	200	210	80	200	280
8....	35	Nil	35	80	120	200
9....	875	"	875	410	70	480
10....	10	290	300	100	160	260

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* This observation is in agreement with that of Bonninger, who found that the addition of large quantities of sodium chloride interfered with the normal secretion and reduced the action of pepsin on proteids.

† Table XVI gives a comparison of the proportions of sodium chloride and hydrochloric present in an exact duplicate of the original test meal, and of the sodium chloride and hydrochloric acid present in the stomach contents removed after an hour and a half. The materials in both cases were unfiltered. The figures represent cubic centimeters of n/10 silver nitrate solution equivalent to the total quantity of chlorine present either in the form of sodium chloride or hydrochloric acid, the estimation being carried out as described in the analytical section. The results observed may be converted into grams of chlorine by multiplying by the factor .00365. The series of experiments was conducted from October 8 to November 10, and at the autopsy of the case on November 25 it was discovered that almost the entire wall of the stomach was infiltrated with carcinoma.

from the stomach walls and a certain proportion of hydrochloric acid actually penetrating in the reverse direction.

The tendency to establish a state of equilibrium not only as regards the total volume and osmotic tension but also between the sodium chloride and the hydrochloric acid is very remarkable in spite of the fact that the infiltrating carcinoma had invaded a great portion of the secreting surface. This tendency is still to be observed in the later experiments carried out less than a fortnight before the death of the patient. In the early experiments the introduction of a large amount of salt was generally associated with the conversion of a considerable portion into hydrochloric acid, the amount of hydrochloric formed in this way greatly diminishing as the disease progressed. On November 3 and 4, when fairly balanced proportions of the sodium chloride and hydrochloric acid were introduced, the state of equilibrium was not very materially altered. On November 5 and 8 very small quantities of sodium chloride and no hydrochloric being introduced in the test meal, sodium chloride and hydrochloric acid were apparently secreted in the proportions of about 1 : 2. November 6, 7 and 10 practically no sodium chloride and large quantities of hydrochloric in excess of the normal introduced in the test meal resulted in a slight loss of hydrochloric acid and a considerable increase in the amount of salt. The high concentration of sodium chloride in the test meal on November 9 was associated with a considerably reduced concentration of salt in the removed stomach contents, and as on October 29 and 30 the introduction of a very large amount of salt with a high osmotic pressure seemed to result in the passage of chlorides through the stomach wall into the system and a slight formation of hydrochloric acid in the stomach either through secretion or the loss of sodium ions, the quantity of acid formed being considerably less than under normal circumstances when moderate or even extremely small quantities of chlorides were administered, as on November 5 and 8.

Through the whole series the tendency of this particular stomach to establish an equilibrium at about 250 units, or .9 to 1 gram of chlorine, is evident. The state of the patient rendered the completion of this series of experiments impossible, and the experimental errors, although reduced to a minimum, introduced sufficient variations to preclude the possibility of working out any mathematical relationships.*

If the test meal was permitted to remain a sufficiently long time in the stomach an osmotic equilibrium representing from .4 to .45° C. was generally established through the interchange of water and salts between the stomach contents and the system. An arrest of the process at the end of an hour to an hour and a half showed, both in cases of high and low osmotic tension of the original test meal, a tendency to the establishment of an equilibrium as near the average as possible, a finding which is more or less in accord with that of Pfeiffer but scarcely in agreement with that of Strauss, who considers that the secretory functions of the cells of the stomach membrane far exceed their tendency to absorb.

It would not of course be possible to base any final conclusions on a single case of this nature, but the observation that a diminished secretion of hydrochloric acid is frequently associated with an increased secretion of sodium chloride justifies the assumption that the failure of the cells to secrete a normal proportion of hydrochloric acid is only in part attributable to a diminished secretory function and may to a certain extent be accounted for as due to faulty or incomplete dissociation of sodium chloride in the blood and the cells concerned, probably due to the influence of sub-oxidation products and toxins produced in, and excreted from the tumor cells. This view tends to support the observation regarding the abnormal decomposition of proteids in cancer stomachs referred to in a previous section. It is also in agreement with the observed diminution in alkalinity and increase in osmotic pressure and sodium chloride content of the blood referred to elsewhere in this report, phenomena which are in all probability directly dependent upon some interference with metabolism resulting in incomplete and faulty oxidation of food stuffs.

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* Similar series of experiments covering shorter periods were carried out on other cases, the same tendency to establish a definite state of equilibrium between NaCl and HCl being observed. In certain cancer cases sodium chloride can apparently almost replace hydrochloric acid in molecular equivalence.

Leo Schwarz, in a valuable paper dealing with the question of chlorine starvation and the alkalinity of the urine, shows that a marked increase of the latter quantity is brought about by introducing sodium chloride and sodium bromide into the stomachs of dogs, bromine apparently functioning as an equivalent for chlorine, whilst nitrates and other mineral salts produce no marked effect on the state of equilibrium of the system.

Reissner concludes that a fairly uniform chlorine content of the stomach is generally to be observed, and that the absence or presence of free hydrochloric and hydrochloric combined with proteids is more or less dependent on the extent to which the original hydrochloric secreted had been neutralized with sodium. He evidently considers that the hydrochloric acid is secreted in any case and subsequently a portion of it neutralized. Our experiments would rather lead us to suppose that a certain proportion, in some cases a considerable amount, of sodium chloride was actually excreted as well as hydrochloric acid; and whilst we have frequently found abnormally large proportions of sodium chloride in cancer cases, as was stated above, other cases of cancer have also been recorded in which practically no hydrochloric acid or sodium chloride was to be found. Whilst in normal stomach contents the phenolphthalein end point, after allowing for phosphates, gives a fairly accurate estimate of the total available hydrochloric acid, as will be seen from Table XVII, this is in no sense true in pathological cases. As was stated in an article on indicators published recently and abstracted in this report, the discrepancies observed can scarcely be accounted for as due entirely to the presence of fatty organic acids and must partially be attributed to the abnormal decomposition of the proteids themselves.

It will be seen that in the first part of this table the available physiological hydrochloric acid estimated by gravimetric methods when added to the acid phosphates is about equal to the phenolphthalein end point, but this is in no sense the case in the cancer results recorded in the next section, although certain of them show very little discrepancy, whilst the big differences observed in the first five cases are in each case partially to be attributed to the influence of lactic acid. There is a residual difference which must be attributed to some type of proteid decomposition. In the last section giving non-cancerous pathological cases it will be seen that certain discrepancies are to be observed, which in view of the fair amount of hydrochloric acid present, can scarcely be attributed to bacterial decomposition.

Table XVII.

Non-Cancer Cases.

CASE	1	2	3	4	5	6	7	8	9	10	11	12
Free and combined HCl	7.7	11.1	9.0	9.4	8.0	5.6	7.1	3.6	2.6	4.1	6.6	6.4
Total phosphates	Nil	Nil	1.5	.6	.3	.4	.8	.8	.4	.4	.2	.6
Sum	7.7	11.1	10.5	10.0	8.3	6.0	7.9	4.4	3.0	4.5	6.8	7.0
Phenolphthalein end point	7.6	10.9	10.6	9.6	8.0	5.9	8.3	4.5	2.8	4.6	6.6	7.1
Difference	—.1	—.2	+.1	—.4	—.3	—.1	.4	+.1	—.2	+.1	—.2	+.1

Cancer Cases.

CASE	1	2	3	4	5	6	7	8
Free and combined HCl	.0	.4	.8	.0	4.0	4.8	2.7	6.2
Total phosphates	.8	.6	.5	.2	1.0	.5	.4	.8
Sum	.8	1.0	1.3	.2	5.0	5.3	3.1	7.0
Phenolphthalein end point	8.8	6.8	4.9	1.1	10.0	5.4	3.3	8.4
Difference	+8.0	+5.8	+3.6	+.9	+5.0	+.1	+.2	+1.4

Pathological Cases Other than Cancer.

CASE	1	2	3	4	5	6	7	8
Free and combined HCl	14.4	8.0	11.0	6.8	7.3	6.1	3.6	2.4
Total phosphates	1.5	.8	1.1	.9	.6	.5	1.0	.4
Sum	15.9	8.8	12.1	7.7	7.9	6.6	4.6	2.8
Phenolphthalein end point	17.0	10.0	15.2	10.1	9.6	4.0	9.0	5.8
Difference	+1.1	1.2	+3.1	+2.4	1.7	—2.6	+4.4	+3.0

IV. *On the osmotic pressure of stomach contents.*—A series of freezing points made on 75 normal and pathological stomach contents gives results varying from .15° to .8° C. lowering of the freezing point. No direct relationship obtains between the freezing point of the stomach contents and the specific gravity, as is the case in urine, owing presumably to the large quantity of starch, etc., usually administered in a test meal. There is not the same difference to observe between the osmotic pressures of the contents of normal and cancer stomachs as is found in the titration values, presumably owing to the increased proportion of salts, sodium chloride and phosphates which are capable of replacing the free acid in maintaining the osmotic pressure in cases in which cancer has already made considerable advance.

The appended Table XVIII gives the comparison of the osmotic pressure of test meals and stomach contents after one and one-half hours, carried out on the same case as that referred to in previous

TABLE XVIII.

Lowering of Freezing Point in Degrees C., Indicating Osmotic Pressure.

Osmotic pressure of meal...	1.27°	1.20	.93	.91	.67	.51	.36	.29	.27	.19
Osmotic pressure of contents after one hour and a half...	.87°	.79	.72	.67	.58	.47	.41	.39	.39	.34

Table XVI. The tendency to establish an equilibrium of osmotic pressure represented by a lowering of the freezing point of about .4° may be observed, all cases in which the osmotic pressure was higher that figure being diminished and when lower increased.*

SUMMARY AND CONCLUSIONS.

1. A series of exact gravimetric determinations makes it apparent that developing cancer of the stomach is generally associated with diminishing secretion of hydrochloric acid in that organ, a point being very frequently reached at which no free mineral acid is to be found.

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*A series of determinations of freezing point and conductivity in comparison with specific gravity, titrations and the amounts of certain chemical constituents of the stomach contents, blood and urine of a series of pathological cases are at present being carried out in this Laboratory and will be reported upon as soon as the material at our disposal is sufficient for that purpose.

2. This decrease in the proportion of hydrochloric acid is frequently associated with an increase in that of the neutral chlorides, and to a certain extent of the phosphates, the evidence inclining rather to support the assumption that the neutral salts are themselves secreted directly from the cells of the stomach wall, and not, as has been assumed by some authors, being produced as the result of interaction between hydrochloric acid normally secreted and the sodium salts of organic acids removed from the disintegrating tumor.

3. When proper precautions are observed in washing the stomach in advance, administering a normal test meal and removing the entire meal after a reasonable period of time has elapsed, no appreciable quantities of lactic acid are to be found except in those cases in which advanced secondary decomposition of the tumor mass has already set in.

4. The secretion of pepsin in normal proportion is maintained to a later stage than is that of hydrochloric acid, a fact which may frequently be demonstrated by the addition of acid to materials derived from a cancerous stomach, when a marked increase in digestion may be noticed on incubation.

5. The proportion of nitrogen present in filtered stomach contents which bears a fairly close relationship to the existing digestive activity is not in any sense proportional to the chlorine secretion.

6. The so-called phosphotungstic filtrate is usually about 20 per cent of the total nitrogen in well-digested mixtures, but may rise as high as 50 and 60 per cent. in cases in which, owing to the presence of abnormally large quantities of hydrochloric or the entire absence of that substance, digestion has taken an abnormal course, and is by no means confined exclusively to cancer cases.

7. The titration of stomach contents by means of phloroglucinvanillin drops, alizarin and phenolphthalein with a separate estimation of acid phosphates, permits in a majority of cases, of a fair estimate of the free hydrochloric to the phloroglucinvanillin drops end point and of the total available hydrochloric acid to the phenolphthalein end point; and the range from drops to alizarin and alizarin to phenolphthalein usually bear a certain relationship to nitrogenous products present in solution.

8. The period from alizarin to phenolphthalein after correcting for phosphates is normally about one-fifth of the total nitrogen, but shows wide variation in certain pathological cases.

9. The influence of the phospates either present in the test meal or secreted by the cells of the stomach may be estimated by a process referred to in a previous publication. (See page 119 of this report.)

10. The tryptophan reaction supposed to indicate cancer of the stomach has not been found to give uniform or conclusive results.

In conclusion it must be said that no individual characteristics in the chemical analysis of stomach contents can be looked upon as having any absolute diagnostic value. Only the intelligent combination of the various analyses with the microscopical examination taken in conjunction with the clinical side of the case can be looked upon as in any sense conclusive. On the other hand, something may be gained by the application of work of this nature to the solution of problems associated with the secretion of acids, etc., in the stomach and the suspension of this function under pathological conditions. It is excessively difficult to prove the existence of any specific toxic bodies in tumors, although from work on blood corpuscles recently carried out it appears probable that such conditions actually exist. In any case the great proportion of the toxic effects observed in cancer cachexia may be attributed to the influence of sub-oxidized products produced under the abnormal conditions of metabolism of the cell. The failure to secrete hydrochloric acid might well be due to some slight interference with the equilibrium of the chlorides, phosphates, carbonates, etc., in the blood serum. A slight increase in the viscosity of the cell membranes, increased difficulty in diffusion of chlorine ions in one direction and carbonate ions and others resulting from the oxidation processes in the opposite direction, might well result in such a disturbance of equilibrium as to lead to the suspension of such a sensitive function as the hydrochloric acid secretion. The observed increase of chlorides in the blood, decrease of alkalinity and increase in osmotic pressure, etc., associated with decreased secretion of certain salts in the urine all point in the same direction, but even when it is established that such conditions actually exist in cancer, it is extremely difficult to differentiate between cause and effect. It is for clinicians, aided by pathologists, and to a certain extent by chemists, to differentiate sharply between that which is cause and that which is effect in phenomena of this nature. Either by interference with the physico-chemical equilibrium of the cell a stimulus to proliferation might be induced or the agent producing the stimulation may at the same time play an important role in the production of the phenomenon referred to above, which might consequently be looked upon as a mere secondary reaction. This is a point upon which it is not possible to throw any considerable light at present.

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ON THE REDUCTION OF STARCH IODIDE SOLUTIONS BY MEANS OF THE AQUEOUS EXTRACTS OF TISSUES.

By G. H. A. CLOWES, Ph. D.

In a recent publication on chemical methods of differentiating hypernephromata from other tumors of the kidney, the statement is made by Croftan that the reduction of a starch iodide solution may be looked upon as characteristic of the extracts of the adrenals and also of hypernephromata, tumors supposedly derived from adrenal tissue. We are not able to confirm this observation of Croftan's. And further, bearing in mind the known decolorizing effect of ammonia and all alkaline solutions, lecithin and unsaturated fatty acids and even the proteids themselves on iodine solutions, it seemed extremely improbable that such a method as that suggested could have any differential value. It appeared to us necessary to submit the method suggested to a series of severe analytical tests before accepting it as a standard.

Croftan makes no exact statements regarding the strength of the iodine solution which he employed. He merely points out that too great a concentration should be avoided in order that a reaction may be obtained. We therefore carried out a series of experiments in which a n/500 solution of iodine and an equally strong solution of sodium-thio-sulphate were employed as standard solutions and a one per cent starch solution as indicator.

The blood serum, blood corpuscles, urine and various organs of a series of animals, as well as post-mortem material from human beings, were tested quantitatively regarding their reducing power. Our procedure was as follows: a given weight of certain tissue was ground in a mortar with a small quantity of sand and distilled water, repeated extracts being made in this manner until the total volume of fluid after filtration was equal to 100c.c. One c.c. of the starch solution was then added to each of three twenty-five c.c. portions; one was titrated directly by means of the dilute iodine solution; a second was first boiled and then titrated; to a third five c.c. of a n/50 sulphuric acid solution were added before titration. In dealing with the majority of extracts it was observed that no very sharp end point was obtainable, but comparable end points might be found in all cases; that is to say, the first end point was that point at which the iodine was no longer completely decolorized, leaving a faint pinkish-blue tinge through the solution for the space of a few seconds. On titrating further an end point was obtained where a distinct blue remained constant for a space of fully a minute. The solution was usually still capable of effecting a further decolorization of two or three c.c. of iodine solution, provided sufficient time was allowed to elapse, but we found that it was most satisfactory for general purposes to employ the second end point indicated above, at which the blue remained constant for about a minute.

The first materials treated in this manner were derived from a case of cancer of the stomach, in which the patient died as the result of a rupture of the stomach wall and in which it was possible to perform an autopsy within three hours after death. The following table represents the reducing power of the extracts of a series of organs, estimated in cubic centimeters of a n/500 iodine solution for each gram of tissue extracted. The first column represents results obtained on direct titration with iodine in the presence of starch in the cold; the second after treatment with a sufficient amount of dilute acid to render the solution acid to all indicators, and the third after boiling sufficiently to coagulate proteids.

It is interesting to note that using material from different portions of the tumor, of the adrenals and of the spleen, in the first case employing a strong and in the second a weak extract, very nearly the same results were obtained, showing that such a method may have considerable value from a quantitative standpoint. An investigation of the figures shows that nervous tissue obtained from

TABLE XIX.*

ORGAN EXTRACTED.		Cold solution.	Acid solution.	Boiled solution.
Nervous tissue		10.0	3.0 to 4.0 (blue)	
Liver		8.8 to 9.0	4.1	7.3 to 7.7
Pancreas		8.0	6.0	
First adrenal		8.0		
Second adrenal		7.8	6.8	
First tumor gland		7.6	7.6	7.6 to 8.0
Second tumor gland		7.5	7.5	
Spleen	a	7.5	5.0	4.2
	b	7.1 to 7.5		
Lung		5.5 to 6.4		
Kidney		5.0		
Omentum		3.0	2.3	
Fat from omentum (alone)		3.0 to 3.5		
Blood serum from heart		1.5		
Pleural fluid		1.1		
Peritoneal fluid		1.05		

the spinal column has the greatest reducing power in the cold, being slightly higher than that of the tumor, adrenal, spleen, liver and pancreas, all of which are comparatively speaking close to one another. The lungs and kidneys come next, followed by the omentum and other fatty materials which show the smallest reduction of all, indicating clearly that the action in question cannot be attributed to any great extent to the interference of fatty acids. An examination of the figures in the second column shows that after treating with acid, whereby all effects attributable to alkaline solutions and to bodies precipitated by dilute acids are eliminated, the tumor material still produces about the same effect, slightly greater than that of the adrenals, whilst all other organs undergo considerable diminution in reducing power, more especially the extract of nervous tissue, which has fallen to less than one-half of its original strength. The effect of boiling is to produce marked diminution in those cases in which a considerable precipitate is produced.

It must be remembered that the material employed in this series of experiments was obtained at an autopsy, consequently, although not more than a few hours elapsed from the death of the patient to the completion of the experiments, certain post-mortem chemical changes might have intervened sufficient to produce a considerable variation in the reducing power of the individual tissues.

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* These quantities represent the volume in cubic centimeters of a n/500 iodine solution capable of being reduced by the extract of one gram of each of the tissues or sera enumerated in the first column.

The next series recorded in Table XX refers to results obtained on treating the extracts of the organs of normal animals with dilute iodine in the presence of starch.

TABLE XX.

ORGAN EXTRACTED.	Cold solution.	Acid solution.	Boiled solution.
Normal guinea-pig:			
Brains	5.8	5.3	5.7
Liver	13.0	12.0	12.0
Adrenals	15.0	14.5	14.5
Kidneys	14.6	14.2	14.3
Blood	1.5	1.5	....
Normal rabbit:			
Kidneys	10.3	10.0	*8.0
Adrenals	9.5	9.0	9.4
Liver	8.7	8.0	*5.1
Lung	5.0	4.7	5.5
Fat	1.0	1.0	1.0
Blood	1.4	1.4	....

* Heavy precipitate on boiling.

It may be observed that the results in this series do not differ very materially from those obtained in the human autopsy case referred to above. The point most worthy of notice is the reducing power of the adrenal extracts which in both cases lies very close to that of the kidneys and liver; in the case of the guinea-pig slightly higher, in that of the rabbit slightly lower than that of the kidneys. In both cases the kidneys, adrenals and liver head the list with a comparatively high reducing power, whilst the blood serum and fatty tissue both show an extremely low reduction, from one-seventh to one-tenth of that shown by the kidneys. Such results afford further proof that any differentiation of adrenal tissue or tissue supposedly derived from the adrenals by means of the starch iodine reaction is, from a quantitative standpoint, entirely out of the question.

We further made extracts of a tumor of the stomach and of the neighboring pepsin secreting cells in order to observe whether there was any material difference in reducing power, but none was observed, as will be seen from the figures 2.2 and 2.4 given by different portions of the tumor and 2.2 and 2.3 by unaffected portions of the stomach wall. The table referring to this case and subsequent cases is omitted owing to lack of space, but it may be briefly stated that provided exactly identical conditions are maintained through any series of experiments, no differentiation by this means is, so far as we have at present been able to observe, capable of practical application. The reduction of iodine by means of solutions which have been treated previously with an excess of weak acid is slightly less than that of the original solutions, whilst energetic boiling resulting in a precipitation of proteids frequently results in a very material reduction of the reducing power. Fatty materials cause very small reduction. This is also the case with blood serum. Only in two cases was a result as high as 2.5 to 3 c.c. n/500 iodine solution by means of 1 c.c. of serum recorded; in one case the blood of a normal individual having an exceptionally high specific gravity and nitrogen content and in the second that of a case of cancer of the breast with metastases. The usual result was in the neighborhood of 1.4 to 1.6.

Blood corpuscles and leucocytes in the form of a clot produced a reduction ranging from 2 c.c. to 3 c.c. and it is proposed when time permits to make a series of determinations on the blood as a whole, of a series of cases, especially those in which a good iodinophilia is obtained with leucocytes. In all these cases treatment with acid invariably reduced the results, but never to an extent of more than fifteen or twenty per cent.

Examination of urine. The figures obtained in this series of experiments vary widely, from two to fourteen c.c. of iodine being required per c.c. of urine, but it is to be observed that perfectly constant results were obtained from the same specimen of urine, whilst that of the same individual might vary considerably from day to day. The highest results recorded were obtained in a case of advanced diabetes from urine containing six per cent of sugar. On the other hand, a urine obtained from a second diabetic case in which the sugar content was also above five per cent, showed results very little in excess of the normal. In a series of cases in which an impaired metabolism might have been expected to result in the secretion of an excess of bodies capable of producing this reduction of iodine in urine, this test was carried out, but it is not possible to distinguish such cases from the normal, and it will be necessary to carry out a good deal more work in this direction before recording any noteworthy results. The effect of adding an excess of acid to urines previously slightly alkaline was not found to be as great as might have been anticipated.

We also tried the effect of adding an excess of iodine solution to any given material the reducing power of which was to be determined, allowing the mixture to stand for a couple of minutes, titrating back the excess iodine with a sodium-thio-sulphate solution of equal strength and adding starch just before the end point was reached. Such a method was also capable of giving duplicate results on the same material provided sufficient care was exercised, but considerable variations between the results obtained by this method and those obtained by the direct method were frequently observed.

Examination of tumors of the kidney. A couple of cases of hypernephrom which had been preserved in formalin were submitted to examination following the directions given by Croftan and at the same time a sarcoma similarly treated and kidney tissue were submitted to an identical treatment. In no case was a reduction obtained equal to that of normal tissue, and the fact must not be overlooked that formalin itself if not removed is capable of exerting a reducing action upon iodine starch solution.

Summary. 1. It is possible, employing a n/500 iodine solution and a freshly prepared starch solution, to titrate the extracts of a large variety of normal tissues provided the greatest precautions are maintained regarding the uniformity of working conditions. The time factor and the stage at which the end point is recognized are most important and should be determined independently by each individual employing the method, such a method having merely a comparative but no absolute value.

2. The reducing power of an aqueous extract obtained from the adrenals is possessed of a strength averaging about the same as that

obtained from the kidneys and liver, the proportions being such that it appears impossible to differentiate between the adrenals and tumors derived from the adrenals and the other organs of the body by Croftan's method.

3. Blood serum, urine, etc., exert a reducing effect upon the solution in question, and it is proposed to accumulate data bearing upon this question with a view to observing whether such a reaction has any value as a means of diagnosing certain pathological conditions.

September, 1903.

Croftan. Virchow's Archiv. Vol. 169.

A NOTE ON THE INFLUENCE EXERTED BY X-RAYS ON THE METABOLISM OF NORMAL ANIMALS.

By G. H. A. Clowes, Ph. D., and W. W. Plummer, M. D.

As a preliminary step to the investigation of the influence exerted by X-rays on the metabolism of cancer animals, a series of experiments was carried out on normal animals as follows: Eight guinea-pigs of the same origin and weighing 350 grams apiece were isolated in large funnels provided with a wire gauze base so as to facilitate the collection of urine and fæces. Four of them were exposed to the action of the X-rays, two for forty minutes and the remaining two for twenty minutes every second day for a period of thirty days, the four remaining animals being kept as a check on the experimental animals. The pigs were stretched on their backs on a board in such a manner that the abdomen was exposed to the direct force of the rays at a distance of eight inches from the poles, a current of 4 amperes and 110 volts being employed. For the first eight exposures merely a small portion of the hair of the abdomen was removed, but for the last five experiments the skin was shaved clean. All eight pigs received the same uniform diet of carrots, with occasional addition of greens. They were weighed at the same time daily and determinations made of the nitrogen of the urine and fæces and of the phosphoric acid, chlorides, indican, etc., of the urine. Blood counts were made from time to time, both before and after the exposures. At the end of the month the pigs were killed and their blood subjected to chemical analysis and tissues to pathological examination. The curves representing the increase in body weight show practically no deviations under the influence of the periods of X-ray treatment. At the end of the thirty days the four normal pigs showed an average increase of 40 per cent, the X-ray pigs an increase of 39 per cent of their original body weight. A scarcely greater difference was to be observed in the nitrogen loss in the urine and fæces, the normal pigs showing an average daily loss per kilo of body weight, amounting to 411 millegrams, whilst that of the X-ray pigs amounted to 394 millegrams. It is interesting to note that this figure is practically double that of the normal human being. The excretion of phosphates and indican followed the same lines as that of nitrogen, being slightly lower in the X-ray pigs than in the normal, which would scarcely be the case had the action of the X-rays on these animals been associated with a destruction of tissue. Blood counts showed fairly uniformly from four to five million reds, but considerable variations in leucocytes before and after exposure to the rays. The following table, based on the average of several determinations, shows in five cases out of six a considerable diminution in the number of leucocytes shortly after the completion of an experiment. The precaution was taken of regulating the diet of the animals in such a manner that the variations can not be attributed to that source. The chemical analyses of the blood gave practically identical results on the normal pigs and the X-ray pigs, the chlorine content averaging .3 to .35 per cent, the nitrogen 2.5 to 2.8 per cent, the only recognizable difference being a slightly greater degree of alkalinity of the serum of the X-ray pigs as compared with that of the normal.

TABLE.

Leucocyte count before and after exposure to X-rays.

Before.	After.
8,800	5,800
5,000	3,700
11,500	6,500
6,500	6,500 (?)
6,100	4,000
7,200	5,600

April, 1903.

A NOTE ON THE QUANTITATIVE ESTIMATION OF PHOSPHATES IN STOMACH CONTENTS.

By G. H. A. CLOWES, Ph. D. (Published in the American Journal of Pharmacy, Vol. 75. July, 1903. No. 7.)

SUMMARY.

It has been shown that the usual method of roughly estimating the effect due to acid phosphates in the titration of stomach contents is incorrect; that one of the three acid affinities of phosphoric acid is saturated at the alizarin end point, and a second at the phenolphthalein end point. Consequently less than one-half of the total effect due to phosphates lies between the diazo (so called) and alizarin end points; while at least an equal effect is exerted on the portion usually looked upon as combined HCl.

A comparatively simple method has been suggested whereby a fairly accurate estimate of the phosphates present and the effect which they exert may be directly determined, dependent

(1) Upon the transition from NaH_2PO_4 to Na_2HPO_4 between the alizarin and phenolphthalein end points;

(2) The transition from Na_2HPO_4 to Na_3PO_4 between the phenolphthalein end point alone and in the presence of $BaCl_2$, whereby the alkaline Na_3PO_4 is converted into $Ba_3(PO_4)_2$ so soon as formed and thus removed from the scene of action.

THE RELATIONSHIP BETWEEN THE FREEZING-POINT DEPRESSION AND SPECIFIC GRAVITY OF URINE, UNDER VARYING CONDITIONS OF METABOLISM, AND ITS CLINICAL VALUE IN THE ESTIMATION OF SUGAR AND ALBUMIN.

By G. H. A. Clowes, Ph. D. (Published in the American Journal of Physiology, Vol. 9. July 1, 1903. No. 5.)

Summary.

1. In normal urine the depression of the freezing point is directly proportional to the specific gravity, and may be determined from the latter by multiplying the figures after the decimal point by 75. (As an example, if the specific gravity is 1.022, $0.022 \times 75 = 1.65$, which is found by experiment to be the depression of the freezing point of such a solution in degrees C.) (See original article, Tables I and II.) This portion of the work is in agreement with the findings of Fuchs.

2. In pathological urines other than those containing sugar and albumin, the same tendency to maintain a constant proportion between the freezing point and specific gravity may be observed, although within rather wider limits than in the case of normal urines. (See original article, Tables III and IV.)

3. The average weight of the molecule or ion in urine may be determined from these figures as approximately 56 to 60, making use of Beckmann's formula,

$$M = E\,\frac{m}{\Delta},$$

where M = molecular weight of substance dissolved; E = a constant having the value of 18.5 when water is employed as the solvent;

m = the quantity of substance dissolved, assumed in this case to be 2.33 grams of substance for each 0.01 of specific gravity; and Δ = the observed depression of the freezing point (0.75° for each 0.01 of specific gravity).

4. In cancer, typhoid fever, and other diseases in which the excretion of chlorides is extremely small, the effect exerted upon the freezing point depression is not so great as might be expected from the removal of such a large proportion of the smaller ions. This tendency to maintain a constant state of equilibrium in the average size of the ions and molecules present in the urine may account for the high retention of the products of sub-oxidation and abnormal metabolism, known to exist in these cases. This view of the case may be advanced as a further argument in favor of the therapeutic administration of chlorides to patients on a milk diet, in order that molecular equilibrium of the urine may be maintained, and a more complete elimination of the products of metabolism effected.

5. In diabetic urines, the quantity of sugar may be very readily estimated by determining the lowering of the freezing point of the urine in question, and calculating the theoretical lowering of the freezing point from the specific gravity. The difference between these two quantities in degrees C., when multiplied by 6, gives a very close approximation of the actual percentage of sugar present in the solution. This factor, 6, calculated on a theoretical basis and subsequently confirmed in practice, is dependent on the fact that the molecular weight of sugar is 180, whilst the average of urine solids is below 60. (See original article, Tables VI, VII, VIII, IX, X, and XI.) These results are not all in accord with the observations made by Fuchs, who arrived at his factor, 10, by purely empirical means.

6. The quantity of albumin may be estimated by determining the specific gravity and freezing point in a urine to which a couple of drops of dilute acetic acid has been added, then boiling in order to precipitate the albumin, filtering and once more determining the specific gravity and freezing point. The second specific gravity is reduced to the same ionic concentration as the first, by multiplying its decimal portion by the freezing point before boiling, and dividing by the freezing point obtained after boiling. This calculated spe-

cific gravity is then subtracted from the original specific gravity determined before boiling, and the difference multiplied by the factor 400, which gives the percentage of albumin. (See original article, Table XII.)

7. In a urine containing both albumin and sugar, the former is first estimated by addition of acetic acid, as above, and the freezing point and specific gravity obtained after boiling are employed in the estimation of sugar.

THE METACHROMATISM OF MAST CELL GRANULES AND MUCIN.

By G. H. A. CLOWES, Ph. D., and ALICE G. OWEN. (Published in the Journal of Medical Research. 1904.)

SUMMARY.

The metachromatism exhibited by the granules of mast cells on staining with 10 per cent polychrome methylene blue may be considered as a simple acid-alkali reaction, the compound formed between the methylenazur of the stain and the granules being sufficiently sensitive to acids and alkalis to function as an indicator in the strict chemical sense of the word.

The granules exhibit a *blue coloration with acids* and a *red with alkalies.*

The tendency to change color on passing from acid to alkaline solutions is exhibited by other constituents in the tissue but to a considerably less marked degree.

Whilst the transition from blue to red with alkalies and from red to blue with acids may be observed whatever the nature of the hardening agent previously employed, it should be noted that formalin hardened material shows a far greater tendency to blue and alcohol and sublimate-hardened material to red, even though the original hardening solutions were perfectly neutral. The ability of formalin to combine with ammonia and amido derivatives may well account for this peculiarity.

Oxidizing and reducing agents produce no marked color changes from red to blue or vice versa when stained mast cells are submitted to their action. Reducing agents tend to decolorize the sections as might be anticipated.

The granules are insoluble in weak acids but readily soluble in weak alkalies. Their tendency to dissolve may, however, be ma-

terially affected by their preliminary treatment with various hardening agents and mineral salts.

The globules of mucin present in goblet cells of the intestinal mucosa stain faintly blue with dilute polychrome methylene blue and show a tendency to red on treatment with acids. On heavily overstaining with concentrated polychrome methylene blue and decolorizing with acetic acid the globules are stained red and on subsequent treatment with dilute alkalies are decolorized or rendered slightly blue. The red color may once more be restored by subsequent treatment with dilute acid, but is not so intense as in the original section. The globules in question are also considerably more soluble in alkalies than are the granules of mast cells.

The leucocytes on staining with polychrome methylene blue show much less polychromatic tendency than the mast cells, retaining both in acid and alkaline solutions a violet to blue color up to the point of decolorization.

Since mast cells take polychrome methylene blue much more readily than does mucin and exhibit a blue color with acids and a red with alkalies, whilst mucin is red with acids and decolorized with alkalies; also since the globules of mucin are far more soluble in alkaline solutions than are the mast-cell granules, it may be concluded that they differ very materially in chemical constitution.

Much is already known regarding the effect exerted on the staining properties of bodies of the type of thionin, methylene blue, etc., on introducing acids or basic groups at various points in the molecule; also regarding the effect of acids, alkalies and oxidizing and reducing agents on the compounds thus formed.

A thorough investigation of the nature of polychrome methylene blue from a purely chemical standpoint, following the lines adopted by Michaelis, would be extremely valuable. It should then be possible, making use of the various constituents of this stain under varying conditions of acidity and alkalinity, to obtain considerable information regarding the chemical nature of the granules and other secretory products present in mast cells, leucocytes, etc., and to compare them with bodies of known chemical composition.

This line of research is one which we propose to take up at the first available opportunity and as soon as the stains in question are procurable.

THE THEORY OF INDICATORS AND ITS BEARING ON THE ANALYSIS OF PHYSIOLOGICAL SOLUTIONS BY MEANS OF VOLUMETRIC METHODS.

By G. H. A. CLOWES, Ph. D. (Published in the American Journal of Pharmacy. 1904.)

In this paper the physico-chemical theory of indicators was discussed in so far as its principles were found to be applicable to the solution of problems involved in the volumetric analysis of physiological solutions.

Practically all indicators are sensitive to strong acids and strong bases. They may, however, be divided into three distinct classes when tested with a series of weak acids and weak bases of the type present in physiological solutions. At one end of the scale are those which in view of an extremely weak acid affinity are particularly affected by weak acids and are, comparatively speaking, indifferent to weak bases, whilst at the other end of the scale are to be found those which are possessed of strong acid or weak basic combining groups which render them very susceptible to the influence of weak bases but more or less indifferent to that of weak acids.

It is further possible to differentiate between a series of two or more acids or bases present in the same solution, provided one can find indicators, the combining affinity of which to form neutral salts, lies intermediate between those of the bodies to be titrated. A good illustration of this mode of differentiation is the recognition of the three acid groups of phosphoric acid, the first one of which coincides with the first alizarin end point, the second with the second alizarin end point, or with the phenolphthalein end point, and the third with the phenolphthalein end point in the presence of barium chloride;

or aspartic acid, for example, the first acid affinity of which is neutralized at the alizarin or phenolphthalein end point, the second only at the end point of Porrier's blue. Carbonic acid affords an interesting contrast to aspartic acid, neither of its acid groups being strong enough to affect alizarin, one only giving an indication with phenolphthalein and both with Porrier's blue. It will thus be seen that in their behavior towards phenolphthalein and alizarin we have a sharp differentiation between the strongest acid affinities of aspartic and carbonic acid respectively.

At the other end of the scale phloroglucinvanillin is sensitive to the amido group present in aspartic acid and entirely indifferent to the acid groups referred to above. Whilst Porrier's blue is absolutely indifferent to ammonia and all weak basic groups, alizarin is sensitive to ammonia but not to amido groups of the type present in glycocol, leucin and tyrosin. Phloroglucinvanillin as an indicator gives a sharp titration of NH_2 groups of this class and one of the NH_2 groups in asparagin. It is unaffected by the second or amido group of asparagin and those of the same type present in acetamid, formamid, urea, etc.

In discussing the effect of albumoses, peptones, etc., on indicators, it was shown that perfectly pure preparations of these substances show a wide range of variation in titration end points, attributable presumably to the influence of weak basic groups present in the molecule; further, that in advancing hydrolysis the range from the lowest to the highest end points was increased, and that this effect cannot be attributable to the influence of phosphates, organic acids and chlorides to the same extent as has been generally supposed.

The comparison of volumetric with gravimetric analyses of normal and pathological stomach contents showed that provided a plain Ewald, or by preference a purely proteid test meal were employed, and the materials examined in the space of three hours, with very few exceptions the phenolphthalein end point gives a fairly accurate estimate of the total available hydrochloric acid present in the stomach, either in a free state or combined more or less feebly with proteids. The influence exerted by phosphates is usually small and may be allowed for by making a separate estimation of the quantity present according to a method suggested by the writer in a previous paper. The end point of phloroglucinvanillin drops used on a warm plate gives a fairly accurate estimate of the actual free hydrochloric acid. The range of titration from the phloroglucinvanillin end point to that of phenolphthalein, after allowing for the influence of phosphates and traces of organic acid may be said to be a function of the basic affinities of proteids and their decomposition products present in the solution. The total range and the end points of intermediate indicators are dependent upon the total quantity of such bodies present in the solution and the extent of their hydrolysis or decomposition, and provided comparable conditions of experiment are employed this range may be said to be fairly proportionate to the peptic activity of the mixture.

Under pathological conditions where hydrochloric acid is absent or diminished in quantity, and peptic digestion fails to take its normal course, especially where organic acids have been produced under the influence of bacteria, the above rules are no longer applicable. The total range no longer bears the slightest relationship to the titration results; chlorides determined gravimetrically are found to be far below the phenolphthalein end point; the nitrogen present in so-called phosphotungstic filtrate rises above the normal, which is from 20 to 25 per cent of the total nitrogen in a well-digested mixture. All these factors point to the abnormal breaking down of proteids beyond the point normally reached in peptic digestion in the stomach, presumably under the influence of bacterial enzymes functioning in a neutral solution with the liberation of additional weak basic and acid groups, as a result of the abnormal hydrolysis.

In tryptic digestion due allowance must be made for the influence of a known quantity of sodium carbonate which may be estimated independently. Both the acid groups of this body are capable of exerting an influence upon Porrier's blue, whilst only one affects phenolphthalein and other indicators such as alizarin or phloroglucinvanillin are entirely unaffected. After allowing for the influence due to carbonic acid it is found that the range of titration increases with increasing digestion, being at the early stages more or less proportionate to the amount of proteid brought into solution as estimated by the total nitrogen, but subsequently increasing in undue proportion to the total nitrogen as digestion proceeds, with the formation of simpler nitrogenous products.

The bearing which work of this nature may have on the solution of certain problems connected with the constitution of proteids and their decomposition products was also discussed. Titrations of normal blood serum showing a wide variation with different indicators which could not possibly be attributed to the inorganic constituents of the serum were tabulated and the necessity of an agreement upon certain specific indicator end points for serum titration emphasized.

The titration results on urine were shown to be fairly dependent on the amount of phosphates present, the small proportion of amido bases and weak acids present exerting a relatively small effect in normal cases. A normal urine should be acid to phenolphthalein and alkaline to alizarin, the end point lying somewhere between the neutralization points of these indicators. In other words, whilst the first acid group of the phosphates should be neutralized, the third acid group should remain free, varying proportions of the second group being neutralized or not according to the state of equilibrium of the system. Naturally in the presence of any considerable quantity of ammonia which in contradistinction to urea exerts a powerful effect upon alizarin and phenolphthalein, the urine would be less acid, or even alkaline to phenolphthalein and more strongly alkaline to alizarin than is normally the case.

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SIXTH ANNUAL REPORT

of the work of the

CANCER LABORATORY

of the

New York State Department of Health

Conducted at the
GRATWICK RESEARCH LABORATORY
UNIVERSITY OF BUFFALO

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For the Year 1904-5

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TRANSMITTED TO THE LEGISLATURE APRIL 1, 1905

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Further evidence of immunity against cancer in mice after spontaneous recovery. By G. H. A. Clowes and F. W. Baeslack.

On the relationship between the rate of growth, age, and potassium and calcium content of mouse tumors (adeno-carcinoma, Jensen). By G. H. A. Clowes and W. S. Frisbie.

Some artefacts in mouse carcinoma. By G. N. Calkins and G. H. A. Clowes.

Recent observations which tend to support the parasitic theory of cancer. By Harvey R. Gaylord, M.D.

REPORT

January 15, 1906.

Dr. Eugene H. Porter, *Commissioner of Health, Albany, N. Y.*

Dear Sir:— It is my privilege to transmit herewith to you the Sixth Annual Report of the Cancer Laboratory, conducted under the auspices of your Department at the Gratwick Laboratory, University of Buffalo.

It gives us great pleasure to report to you an unusually prosperous year in the researches which we are conducting. We are enabled at this time to present to you the completed reports covering the most interesting and significant subject of the spontaneous cure of cancer in animals, a number of preliminary communications on the evidence of immunity to cancer and several interesting papers relating to the growth of cancer in animals, considered from a biological chemical standpoint.

Our reseaches for this year, as recorded in the articles above enumerated, have been rewarded by the demonstration of the fact that *cancer is in principle a curable malady.* In the light of the prevailing pessimism as to the outlook for a cure for cancer, other than surgical intervention, this becomes a fact of the first importance. The preliminary reports already published elsewhere and collected in this report, relating to the immune factor in cancer, point out one direction in which investigation may lead to fruitful results.

The staff of the laboratory for the past year shows but slight changes. With the approbation of the previous Commissioner of Health a new arrangment was entered into during the past year, by which Dr. Park transferred the position of Director of the laboratory to the undersigned, he assuming the position of Chairman of the Advisory Committee of the laboratory. The present organization of the laboratory is, Advisory Committee: Dr. Roswell Park, Chairman; Dr. Charles Cary, Staff; Dr. Harvey R. Gaylord, Director; Dr. G. H. A. Clowes, Chemist; Dr. Gary N. Calkins, Biologist.

F. W. Baeslack	Assistant in biology and histology.
Damon Averill	Assistant in photo-chemistry.
——— ———	Assistant in chemistry.
——— ———	Assistant in microscopy.
Clara A. Maclay	Secretary.
F. A. Payne	Janitor.
M. Weber	Laborer.
E. Kempkes	Laborer.
Julius Huse	Laborer.
E. Heppert	Laborer.

The organization of the laboratory is such that the three principal departments — the pathological, the chemical, and the biological — are conducted as independent departments, the heads of which work in conjunction with one another. The working staff of the laboratory meet regularly and discuss the work, and once a month confer with the Advisory Committee as to its progress. The general welfare of the laboratory is cared for by the Advisory Committee, all working in conjunction with yourself.

The work of the past year has been more fruitful of results than at any time since the laboratory was established. The experimental work begun during the preceding year, for the original material for which we have to thank Professor Jensen of Copenhagen, who was kind enough to place at our disposal two of his cancer mice, has been developed and has now reached a point where practical and suggestive results are being constantly attained. The development of this work has placed a steady drain upon the finances of the laboratory and we find ourselves, at the conclusion of this our most successful year, at a point where the present appropriation is not sufficient to permit us to develop the experimental work to its legitimate limits. There are at present in the laboratory under daily attention and observation 3,000 white mice and 300 white rats. The cost of these animals and their food and attendance during the past year have taken nearly a quarter of our entire appropriation. For this reason we have had to retrench considerably. We have been unable to appoint a chemical assistant, who is greatly needed in the chemical department, and the increased demands upon the pathological department call for the addition of at least one more assistant in this department.

The last year may be spoken of as one of great advances in cancer research, not only in this laboratory but in several others in Europe. Work of this nature with small animals is being conducted on a large scale in the laboratory of the College of Physicians and Surgeons in London, in the Imperial Laboratory for Cancer Research in Berlin, in the department for the investigation of cancer in Professor Ehrlich's Laboratory in Frankfurt, in the Pasteur Institute in Paris, and in several other university laboratories. The certainty of results in this work is largely dependent upon the number of animals which it is possible to employ, and this again is merely a question of expenditure. In this connection we are at a great disadvantage when the number of animals which we have been able to utilize with our appropriation is compared with the number which is being used in England. We have had to date 3,479 mice under observation. In the English laboratory they have, up to the period of their present annual report, recorded the use of 30,000 mice, and although we are not definitely informed, the scope of Professor Ehrlich's work is several times larger than ours, enabling him to work with ten different strains of cancer in the white mouse.

In spite of our disadvantages in this connection I think we may properly call attention to the important papers contained in the present report of the laboratory. Our success during the coming year will depend largely on the support which we receive from the Legislature in the matter of our appropriation. The expenses of the laboratory during the past year are as follows:

1905.		
Sept. 30.	Stock and material	$2,044 86
	Equipment	1,221 25
	Expense	3,337 74
	Salaries	10,800 50
		$17,404 35

1904.			
Sept.	30.	Balance 1903-4	$1,458 09
		Appropriation, 1904-5	15,000 00
		Balance September 30, 1905	946 26
			$17,404 35

From this it will be seen that although we had a balance at the beginning of the year of $1,458.09, we still exceeded our appropriation by nearly $1,000, which we met by drawing on our present appropriation.

In February of 1905 we had the honor of a visit from several members of the Legislature. These gentlemen were so favorably impressed with the economical manner in which the laboratory was conducted that when we explained to them that we required an extra appropriation of $3,000 for this year to enable us to undertake certain lines of work, an item was introduced in the Supplemental Supply bill carrying with it an appropriation for $3,000. Unfortunately this item was stricken from the bill by the Governor, with the result that beginning this year with a deficit of $1,000, we will find ourselves at the end of the present year with a shortage of at least $2,500. During the past year there has developed in the laboratory in some white rats a type of cancer known as sarcoma. It is of paramount importance that we should have the funds to carry on extensive inoculation experiments with this type of tumor. With the present appropriation we shall not only be unable to enter this field of very important research, but we shall have to limit the scope of the present very fruitful lines of experimentation which we are conducting.

I wish to call your attention to the fact that the salaries in this laboratory are small compared with the salaries paid in other institutions of a similar nature. It is impossible for us to maintain the necessary working staff with less outlay than at present. The scope of the experimental work which we are conducting should be broadened and increased if we are to make the progress which, in the interests of humanity and from the standpoint of economy for the State, we should make, and if we are to succeed in fulfilling the purposes for which this laboratory was established.

As Dr. Park proposed in his report of last year, it is extremely desirable that we should have funds for the purchase of two or three simian apes (chimpanzees, gibbons, orang-outangs). These animals have been shown by recent investigations to be so closely allied to the human race that for experimental purposes they are the equivalent of human beings. It is impossible that we should be able to make progress in the direction of the application of the facts which we are steadily developing regarding cancer in the lower animals, unless, through the medium of these apes, we are able to test the applicability of these principles to the treatment of cancer in human beings. The appropriation which was passed by both the House and the Senate last year for this purpose, $3,000, would be sufficient. To meet the deficit of the present year and to provide us a safe margin for the coming year, on our experimental work, we should have at least $5,000. We would therefore ask you to approve and request of the Legislature that our appropriation for the year 1906 should be increased to $23,000. With this sum at our disposal we may look forward to a year of great significance in the development of our work.

This laboratory is the first one in the world established for the special investigation of cancer, and it is entirely proper that the State of New York should have the credit of leading, or at least maintaining its position in the forefront of this line of research. So far as the coming year is concerned, this is purely a question of the funds which the Legislature will place at our disposal. I think we may fairly call attention to the significance of the articles contained in this report and to express the belief that the results obtained during the past two years are quite sufficient to demonstrate the wisdom of the establishment of this institution and to justify the expenditure entailed.

The importance of cancer as an economic disease has in no way diminished during the past year. The statistics for the last two years are as follows:

	Cancer.
Year ending December 1, 1905	5,976
Year ending December 1, 1904	5,713
Increase	263

	Tuberculosis.
Year ending December 1, 1905	14,050
Year ending December 1, 1904	14,166
Decrease	116

It will be seen that whereas the number of cancer cases during the past year has shown a ratio of increase of 4.6 per cent., tuberculosis is practically stationary, having actually decreased .8 per cent. from the figures of 1904. I will call your attention to the fact that the study and treatment of tuberculosis receives from the State of New York an appropriation of $85,000, whereas cancer, which is only second in importance and claims nearly one-half as many useful members of the community, receives less than one-third as much in the appropriations of the State.

Since the establishment of this laboratory a number of similar researches have been undertaken in other countries and, beginning with the development of the experimental method by the use of small animals, a new era in cancer research has been inaugurated. It may fairly be stated that we have learned in the last two years more about cancer than in the preceding twenty-five, and I think we may safely face the future with the conviction that the pursuit of the investigation of cancer along the present lines will, in a reasonable length of time, lead us to an understanding of this disease which will enable us first of all to check its constantly encroaching ravages, and to hold out the prospect of dealing with it from a sanitary standpoint at a not too distant period, with almost as much success as has attended the campaign made against tuberculosis, which has yielded from the economic standpoint such splendid results.

On the part of the staff of the laboratory I take pleasure in expressing to you our appreciation of your constant sympathy and support during the past year. I can assure you that all connected with the Laboratory enter upon the approaching year with greater satisfaction and more sanguine expectations than at any time since the establishment of the Laboratory.

I am, very respectfully yours,
HARVEY R. GAYLORD, M.D.,
Director

Preliminary Report on the Presence of an Immune Body in the Blood of Mice Spontaneously Recovered from Cancer (Adenocarcinoma, Jensen) and the Effect of this Immune Serum Upon Growing Tumors in Mice Infected with the same Material.*

By Harvey R. Gaylord, M.D., G. H. A. Clowes, Ph.D., and F. W. Baeslack, B.A.

In the latter part of February one of us (Gaylord) visited Copenhagen and received through the courtesy of C. O. Jensen, Professor in the Veterinarian and Agricultural High School in Copenhagen, two white mice with actively growing tumors, inoculated from a strain of mice infected with adenocarcinoma, described by Jensen in the *Centralblatt für Bacteriologie,* Erste Abt., vol. xxxiv. These mice were brought successfully as far as New York, but between New York and Buffalo both died. One of them was used on the day following death for the inoculation of twelve mice. The second mouse was placed on ice and on the third day following death the tumor was used to inoculate twenty-five white mice obtained from a source outside of Buffalo. All of the inoculations from the first mouse were unsuccessful. From the second mouse 60 per cent. of the inoculations were successful, thus giving us the material for further inoculation. From that time to the present we have succeeded in having constantly on hand a number of infected mice in various stages of the disease. Our percentage of "takes" in susceptible mice varied from 20 to 70 per cent.

In September and October we noted, for the first time, in a number of mice, that the tumors which had grown to a demonstrable size, ceased growing and underwent a form of spontaneous retrogression which terminated in the disappearance of the tumor without recurrence. Shortly after the observation of these facts a combination of circumstances resulted in our having, for a period of time, but a few available tumor mice. For this reason certain experiments in immunity which had been previously commenced were suspended. In the latter part of November the supply of mice was sufficient to resume the immunity experiments. In the meantime we had succeeded in getting a large number of mice in which the transplanted tumor grew with unusual rapidity and great virulence, showing no tendency to retrogression. For the purpose of determining whether the blood of the mice that had recovered spontaneously possessed any immunizing qualities, a series of experiments was carried out with the following results:

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* Reprinted from the Medical News, Jan. 14, 1905.

The blood-serum of mice which had recovered spontaneously from tumors possessed a power, when injected into mice infected with growing tumors, of inhibiting the growth of large tumors and causing the retrogression of smaller tumors, leaving the animal possessed of an immunity which prevents recurrence of the growth. The degree of immunity in the mice thus far tested varies within considerable limits, the most marked illustration of its activity being found in one mouse whose blood-serum injected in a single dose of .2 c.c. caused the rapid retrogression and entire disappearance of two tumors in one animal and one in another, all of which were as large as peas, in the space of three days. The same serum injected into a mouse with a tumor the size of a small cherry (about two grams), caused a noticeable reduction in size of the tumor, which remained stationary for ten days, when an operation for the removal of a portion of it resulted in a return of activity to the growth. The latter part of this experiment will be dealt with in our final publication.

All of these experiments were controlled with mice inoculated at the same time, the tumors of which were smaller than those of the mice treated with the immune serum. These control mice received doses of normal mouse serum equal in volume to the doses of immune serum referred to above. In every case the tumors in the control mice developed rapidly and led in the course of three or four weeks to the death of the animal. In spite of the fact that the control tumors were invariably smaller than those used for the immune serum at the commencement of each experiment, in the course of a week or ten days the control tumors were found to be larger, and up to the date of making this announcement, while several control mice have died of their tumors, not a single mouse treated with immune serum has so far succumbed.

In those cases in which the tumor was too large or the immune serum too weak to effect a cure, the marked retardation in the development of the tumor was always associated with a diminution in the cachectic symptoms invariably exhibited by the tumor mice in the last stages.

The second stage of our work has shown that the mice cured by injection with the immune serum referred to above possess in like manner active immune qualities in their serum, which thus far have proved capable of causing the disappearance of small tumors and the inhibition of larger ones.

The test tube experiments carried out to determine the nature of this serum, and such information as we have been able to obtain as to the mechanism of its activity from sections of tumors inhibited and cured, lead us to the conclusion that in all probability we are not dealing with a cytolytic serum. We wish, however, to reserve our opinion until we have accumulated more data. Sections of tumors which have undergone partial spontaneous retrogression show that the changes in the epithelium are closely allied to simple atrophy. The connective tissue stroma of the tumor increases greatly in amount, and in the last stages nothing is found but a connective tissue nodule with occasional pseudo-giant cells produced by coalescence of the remaining rests of epithelium, similar to those described by Becker and Petersen, as an evidence of spontaneous healing at the margin of cancer in human individuals. The changes in the tumors inhibited in growth show about the periphery a marked increase in the connective tissue stroma with extensive round-celled infiltration, characteristics which are not found in the growing tumors. At the margin of these tumors one finds an actual disintegration of cancer nests, atrophy of the epithelium, giant-cell formation and final disintegration. The remains of the small tumors, which have disappeared under the influence of the immune serum, consist of minute masses of connective tissue which in the later stages present the characteristics of ordinary organizing connecting tissue. A tumor which received but one injection of immune serum from a mouse cured of a small tumor by the activity of serum from a spontaneously cured mouse, and which decreased from the size of a small pea to that of a grain of rice within thirty-six hours, and which remained stationary for ten days, was found on examination to consist of a mass of newly-formed connective tissue surrounding the remnants of atrophied and disintegrating epithelium. In this case the evidence of disintegration of epithelium was greater than that found in the tumors spontaneously recovered. A description of the histological characteristics will show that the changes in the epithelium are similar in principle, differing only in the rapidity of the process. The changes found in the spontaneously cured tumors and in those inhibited or cured with the immune serum correspond to the changes already described by several authors as an attempt at spontaneous cure in human cancers.

A review of the literature shows that authentic cases of spontaneous cure of cancer in human beings are not unknown, and the correlation of our histological findings with those already noted in man lead us to the conclusion that a similar immunity undoubtedly exists against human cancer. Although our work thus far has shown us that great difficulties will undoubtedly be encountered, it is perhaps not too much to hope that a careful analysis of the facts obtained in our experimentation on mice may ultimately lead to a practical application of these facts with a solution of the question of the curability of cancer in human beings.

No. 31.*—Further Evidence of Immunity Against Cancer in Mice After Spontaneous Recovery.[1]

By G. H. A. Clowes, Ph.D., and F. W. Baeslack, B.A.

In this communication it is proposed to summarize briefly the results of certain immunity experiments which constitute a part of a general investigation being carried on at the present time on the Jensen tumor mice by the staff of this laboratory. In previous publications[2] it was shown that the blood serum of spontaneously recovered mice exerted an effect upon growing tumors in other mice. It was pointed out, however, that the method employed (although affirmed by pathological findings) failed to give proof of the existence of a specific immune body because of the impossibility of determining the course of development which a tumor would have taken if untreated. We have been led, therefore, to adopt two other methods in the hope of demonstrating experimentally the existence of an immune body:

1. The reinoculation of spontaneously recovered mice.
2. The admixture of cancer material previous to inoculation with the serum of recovered mice, the results being controlled by mixing material from the same tumor with normal serum or salt solution.

Up to the present time (June, 1905) about 1,600 mice have been employed in this laboratory in various experiments relating to the transplantation and development of the Jensen tumors. If we exclude from this number those which have been employed for

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* Reprinted from The Medical News, Nov. 18, 1905.

[1] Received for publication, July 15, 1905.

[2] Preliminary report on the presence of an immune body in the blood of mice, spontaneously recovered from cancer (adenocarcinoma, Jensen), and the effect of this serum upon growing tumors in mice infected with the same material. By H. R. Gaylord, M.D.; G. H. A. Clowes Ph.D., and F. W. Baeslack, B.A., *Medical News, January* 14, 1905.

Preliminary communication regarding an immune body capable of inhibiting the development of cancer in mice (adenocarcinoma, Jensen). By G. H. A. Clowes, Ph.D., Bull. Johns Hopkins Hospital, vol. xvi, No. 169, April, 1905.

experiments in which filtration, the use of high temperatures, chemicals, etc., might preclude the possibility of development, and those which, on account of infection or other causes, have died shortly after inoculation, it may be said that about 1,100 mice have yielded about 350 tumors, of which number about 300 have developed and caused the death of the mice, the remainder having recovered spontaneously.

Owing to extreme variability in the time of development of tumors it is often difficult to distinguish transient inflammatory masses from true tumors. The difficulties have been largely overcome, however, by the adoption of a system of diagrams or "charts" of the tumors made at stated intervals and kept as permanent records. The study of over 300 series of such diagrams, covering in some cases periods of several months, makes it apparent that whereas early inflammatory masses frequently make their appearance shortly after inoculation, they differ from true tumors, by either being absorbed, or ulcerating out in the course of two or three weeks. A true tumor, on the other hand, develops somewhat later, generally from two to four weeks after inoculation, and is, as a rule, more consistent than the inflammatory masses referred to above. The speed of development of a tumor is subject to enormous variations, the most rapid having shown a weight of ten or twelve grams within twelve days of their first appearance, others having required several months to reach a weight of two or three grams. In some cases an early developing tumor remains stationary for a considerable period and subsequently either goes on to full development or retrogresses and eventually entirely disappears, nothing but a small residue of connective tissue being found at autopsy.

It is very difficult to determine exactly what constitutes a spontaneous recovery from a true tumor. All early inflammatory swellings are, of course, excluded, as, also, small indefinite grains which make their appearance for a short time only. We have for the present adopted as a standard the presence of a growing tumor not less than three weeks after inoculation, reaching at some period of its development a volume of twenty cubic millimeters. The recovery from such a tumor may be said to constitute a true spontaneous recovery, whereas the recovery from smaller tumors must be designated as somewhat doubtful.

1. *The Reinoculation of Mice Spontaneously Recovered.*— Up to the present date thirty mice which have recovered spontaneously of genuine tumors or have been cured of their tumors by serum injection have been reinoculated, and of this number ten have been inoculated for the third time. In no case has a tumor developed as a result of reinoculation on a mouse which had previously recovered from a true tumor, as designated above, whereas mice which had either shown nothing on the first inoculation or only a rapidly subsiding inflammatory process have produced tumors.

One group, 209, including eight mice, of which seven — A, B, C, D, E, F and H — were reinoculated, is of particular interest and may be selected as an illustration of this point. On March 5th this group was inoculated for the first time with normal cancer macerated with salt solution in the proportion of 1:3, each mouse receiving ½ c.c. subcutaneously in the back of the neck. On March 31st A had a tumor the size of a pea which had first been recognized on March 16th. On the same date (March 31st) E and H had tumors of slightly smaller size but fully equal to the requirements enumerated above. An examination of the chart book for this period shows that in the case of these three mice, A, E, and H, the tumors which first appeared about ten days to a fortnight after inoculation had reached a maximum about March 31st, twenty-six days after inoculation, and had then steadily diminished in size until between April 21st and April 30th they had all three practically disappeared.

Of the five remaining mice of this series, G died on April 10th, and the four mice, B, C, D, F, never showed true tumors. The seven survivors were reinoculated June 2d, three months after the first inoculation, with a tumor which was more virulent than that previously employed. An examination of the chart books on the day of writing this paper (June 26th), twenty-four days after the second inoculation, shows that A, E, and H have no tumors, B, C, and F have fair-sized growing tumors, and D is dead. It will thus be seen that the three mice which recovered from true tumors on first inoculation now possess apparently an immunity against further inoculation, whereas the three which failed to show tumors on the first inoculation now succumb to the more virulent material.

Lot 209 was one of several series reinoculated on or shortly before June 2d. In all, sixty-six mice, fifteen of which had recovered spontaneously from true tumors, and twelve from questionable tumors, were reinoculated on this occasion. At the date of writing this paper, twenty-six days after inoculation, some seventeen tumors are found to be developing in the entire series, some of which will doubtless recover spontaneously. Fifteen cases of unquestionable recovery show no tumors. In addition to these, one tumor is developing on a mouse which originally recovered from a tumor which fell slightly below the requirements enumerated above, and two other tumors are to be found on cases of questionable spontaneous recovery in which small grains made their appearance intermittently and for short periods. In an entirely different series a tumor occurred on reinoculation of Mouse 179 E, which three months previously had recovered from a small, soft tumor about the size of a buckshot, but since the tumor in question was less than 3 mm. in diameter and only appeared for two or three days, we considered ourselves justified in excluding it from the cases of true spontaneous recovery and including it among those of questionable tumors. We have unfortunately been compelled to use a large number of the best spontaneous recoveries for the experiments which will be dealt with under the second head, but as all back series are now being reinoculated, we hope to be able to report on a much larger number of experiments of this nature in the course of the next six months.

2. *The Admixture of Cancer Material Previous to Inoculation with the Serum of Recovered Mice.*— Our early experiments having made it appear probable that the serum of spontaneously recovered mice, when used in relatively large doses, possessed immune properties capable of interfering with the development of small tumors on direct injection, it seemed advisable to observe the effect exerted by a serum of this nature when admixed with macerated tumor materials previous to inoculation. For this

TABLE OF RESULTS.

NORMAL SERIES.

SERIAL NUMBERS.	Date of inoculation.	Number of mice used.	Rapidly growing tumors.	Slowly growing tumors.	True spontaneous recoveries.	Questionable spontaneous recoveries.	Survivors two months after inoculation.
192, 193	February 22	66	24	1	10	14	30
195, 197	February 24						
198, 199, 200	February 28						
207, 208, 209	March 3	24	0	1	5	4	13
243, 246, 247, 248	March 22	20	5	1	0	2	10
300, 303, 304, 307	April 26	23	4	6	1	2	16
Total	....	133	33	9	16	22	69

IMMUNE SERIES.

SERIAL NUMBERS.	Date of inoculation.	Number of mice used.	Rapidly growing tumors.	Slowly growing tumors.	True spontaneous recoveries.	Questionable spontaneous recoveries.	Survivors two months after inoculation.
194, 196, 201	February 22 February 24 February 28	29	5	2	3	10	22
201, 205, 206	March 3	24	0	1	3	3	19
244, 245	March 22	14	0	0	0	3	5
301, 302, 305, 306	April 26	22	0	3	0	2	16
Total	....	89	5	6	6	18	62

SUMMARY.

	Normal Series.		Immune Series.	
Total number of mice employed	133	Per cent.	89	Per cent.
Rapidly-growing tumors	33	24.8	5	5.6
Slowly-growing tumors	9	6.8	6	6.7
Total tumors	42	31.6	11	12.3
True spontaneous recoveries	16	12.0	6	6.7
Questionable spontaneous recoveries	22	16.5	18	20.0
Total spontaneous recoveries	38	28.5	24	26.7
Survivors after two months	69	51.9	62	69.7

purpose it was necessary to obtain a serum of sufficient virulence to produce an effect when distributed over a large number of cells, and to use a tumor sufficiently attenuated in its virulence to be susceptible to the influence of such a relatively diluted serum. It had been found by previous experience that young, small, actively growing tumors do not, on injection into fresh mice, produce tumors with any degree of certainty, and that for successful transplantations it is essential that a tumor should be, at least in part, necrotic. It had further been observed that large aggregates of cells produce tumors with greater facility than does material from which the connective tissue had been removed by fine rakes or by filtering through coarse gauze.

At the time at which these experiments were commenced we had not a sufficient number of carefully charted spontaneous recoveries at our disposal to afford us any idea regarding the conditions under which the highest degree of immunity might be expected to develop. In some cases mice which had simply recovered from an inflammatory process were probably mistaken for true spontaneous recoveries and used as such, with more or less negative results, which are, however, included in the experimental series tabulated below. Another point regarding which we have at present very little information, concerns the period after spontaneous recovery, when the maximum degree of immunity may be found, and on account of the extreme delicacy of the reaction, it is to be expected that great difficulty will be experienced in throwing light on this point.

In order to compare the relative effect produced by the serum of suitable spontaneous recoveries, and of comparable normal mice, the following experimental procedure was adopted: A suitable tumor was removed and admixed with salt solution in a sterilized mortar in the proportion of 1:3. Three equal portions were measured into three sterilized mortars. To the first normal serum was added; to the second, an immune serum, and to the third, salt solution. The mixtures were then thoroughly stirred and allowed to stand from one to two hours and subsequently uniform doses were injected into three series of mice. The difficulties enumerated above, more especially that of obtaining absolutely comparable uniform materials on account of the presence of small aggregates of cells, materially influenced the results and may have had something to do with the appearance of some tumors in the immune series. However, an inspection of the accompanying table shows a distinct advantage in favor of the immune series.

A summary of the experiments of this nature, carried out from February 22d to April 26th, shows that of eighty-nine mice employed in the immune series, 5.6 per cent. developed large, rapidly-growing tumors, and 6.7 per cent. slowly-growing tumors, giving a total of 12.3 per cent.; whereas of the 133 mice employed in other series, including normal serum, cachectic serum (derived from dying tumor mice), and salt solution, 24.8 per cent. showed large, rapidly-growing tumors, and 6.8 per cent. slowly-growing tumors, giving a total of 31.6 per cent. There appeared to be rather more true spontaneous recoveries in the normal series than in the immune series, but the figures in question, 6 in 89 and 16 in 133, are rather too small for any positive conclusions.

It is of particular interest to note that two months after inoculation 70 per cent. of those used in the immune series are still living, while 51.8 per cent. only of the normal and other series are living. To the actually recorded figures for tumors should be added four mice in the immune series and one in the normal series, which at autopsy showed small metastases in the lungs and other organs, but no external tumor.

We are inclined to believe that normal serum produces a slight effect as against salt solution, but the differences are too small to be considered at the present stage of our experiments. An inspection of the chart books of certain groups in which the immune serum produced the most marked effect shows that not only was the number of tumors in the immune series smaller and the number of survivors larger than in the normal series, but also tumors made their appearance rather later in the immune series, a fact which is indicated in the tables by the relative proportions of five quick-growing and six slow-growing tumors in the immune series, as against thirty-three quick-growing and nine slow-growing tumors in the normal series.

It is quite conceivable that a relatively feeble immune serum, when brought into contact with large aggregates of cells, is absorbed by those on the surface and fails to penetrate to the interior of the mass. The difficulty of producing tumors with small quantities of material or with single cells makes it impossible, at present at least, to eliminate this source of error.

Whereas in the early series in which the macerated cancer materials were simply admixed with salt solution, mice were never known to recover spontaneously from tumors beyond the size of a large pea, there have been eight cases of recovery from remarkably large tumors in some of the later series in which the materials had been admixed with either normal or immune serum previous to inoculation. This is a point on which future experiments should throw further light.

Summary.— (1) Evidence has been afforded that mice which have recovered from cancer possess an active immunity against further inoculation. (2) By mixing cancer materials previous to inoculation with the serum of such spontaneously recovered mice, and comparing its effect with that of cancer materials from the same source mixed with serum from normal mice, it has been shown that the former exerts an inhibiting effect, and that the percentage of tumors is much smaller and the percentage of mice surviving the treatment is larger in the immune than in the normal series.

Although we fully realize the fact that many more experiments must be undertaken, and that more positive results must be obtained before the presence of an immune body in mice spontaneously recovered from malignant tumors can be proved, we nevertheless feel justified in announcing again that all evidence thus far obtained in this most difficult and dimly-lighted field of research is distinctly in favor of the hypothesis.

No. 32. On the Relationship Between the Rate of Growth, Age, and Potassium and Calcium Content of Mouse Tumors (Adenocarcinoma, Jensen).*

By G. H. A. Clowes and W. S. Frisbie.

This communication deals with one phase of a general investigation being carried out at the present time on the Jensen tumor mice by the staff of this laboratory. The original tumor from which this series has been derived was discovered and successfully transplanted through several generations of mice by Professor Jensen of Copenhagen, through whose courtesy a couple of mice were placed at the disposal of the staff of this laboratory in February, 1904. The mice were brought to Buffalo by Dr. Gaylord, the tumors removed, admixed with normal salt solution in the proportion of 1:3, and injected subcutaneously into a couple of series of normal mice. Several tumors developed in one of these batches and afforded material for further transplantation and experiment.

Up to this time about 1,600 mice have been employed in this laboratory in the experimental work directed toward the determination of the conditions influencing the development of these tumors. Excluding those which have been employed for experiments in which the use of filtration, high temperatures, chemicals, etc., previous to inoculation, might be expected to interfere with the normal development of the tumor, also those cases in which some type of infection or other accident led to the death of mice shortly after inoculation, it may be said that approximately 1,100 mice have yielded about 450 tumors, of which number over 300 have developed and caused the death of the mice.

The results of certain preliminary immunity experiments affording evidence of the existence in spontaneously recovered mice of forces antagonistic to the development of the tumors are

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* Reprinted from the American Journal of Physiology. Vol. XIV, Sept. 1, 1905, No. III.

to be found in the communications from this laboratory, Nos. 27, 28 and 31.[1]

Previous to December, 1904, the number of tumors at our disposal was too small to permit of their being employed for the determination of mineral constituents, and experiments made with a view to isolating and identifying certain organic constituents had to be abandoned for the time being, on account of the extremely small size of the tumors, which seldom reached ten grams, most frequently weighing from three to seven grams at the time of the animal's death. Recently, however, the yield of tumors has increased much more rapidly than was required for experimental purposes. Tumors of mice which die or which show a leucocyte count over 18,000 are never employed for reinoculation, and these tumors have afforded ample material for the chemical investigations which are the main object of this paper.

A few analyses of human tumors have been published by Beebe, who was, however, compelled to make use of materials of various origins, which can scarcely be said to afford an accurate basis for comparison; for example, the ash of an angio-sarcoma of the leg being compared with that of a round-celled sarcoma of the shoulder. The author found that the former tumor showed signs of profound degeneration accompanied by a low potassium and a high calcium content, whilst the latter, without apparent degeneration, showed a high potassium and a low calcium content. Owing to the difficulty of comparing tumors of such diverse nature, generalizations as to age and potassium and calcium content are at least open to criticism.

The mouse tumors with which we are dealing, on the other hand, offer particularly favorable opportunities for such a comparison, being easily controlled and examined under varying conditions of virulence, age, and necrosis. They present, however, one objection in that they are extremely small, the errors of working being thus accentuated.

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[1] No. 27. Preliminary report on the presence of an immune body in the blood of mice spontaneously recovered from cancer (adeno-carcinoma, Jensen), and the effect of this immune serum upon growing tumors in mice infected with the same material. By Harvey R. Gaylord, M.D., G. H. A. Clowes, Ph.D., and F. W. Baeslack, B.A. *Medical News*, January 14, 1905.

No. 28. Preliminary communication regarding an immune body capable of inhibiting the development of cancer in mice (adeno-carminoma, Jensen). By G. H. A. Clowes, Ph.D. Johns Hopkins Bulletin, vol. xvi, No. 169, April, 1905.

No. 31. Further evidence of immunity against cancer in mice after spontaneous recovery. By G. H. A. Clowes, Ph.D., and F. W. Baeslack, M.A.

In the course of the last six months 100 tumors have been analyzed, the sodium, potassium, and calcium being determined if present. Magnesium has never been found in appreciable quantities. Several (ten to fifteen) of these tumors should be excluded on account of their extremely small size, one to two grams of material yielding, for example, only 0.01 to 0.03 grams of ash, a quantity too small to afford a reliable estimate of the potassium and calcium elements which seldom exceeded 10 per cent. of the total ash. In such cases, however, it has frequently been possible to determine whether a given constituent was absent or unrecognizable, present in traces only or in considerable quantities. All the results obtained have been recorded in tabular form, including full data regarding the mouse, the tumor, the origin of the latter, etc., in order to facilitate future reference (Tables II to X).

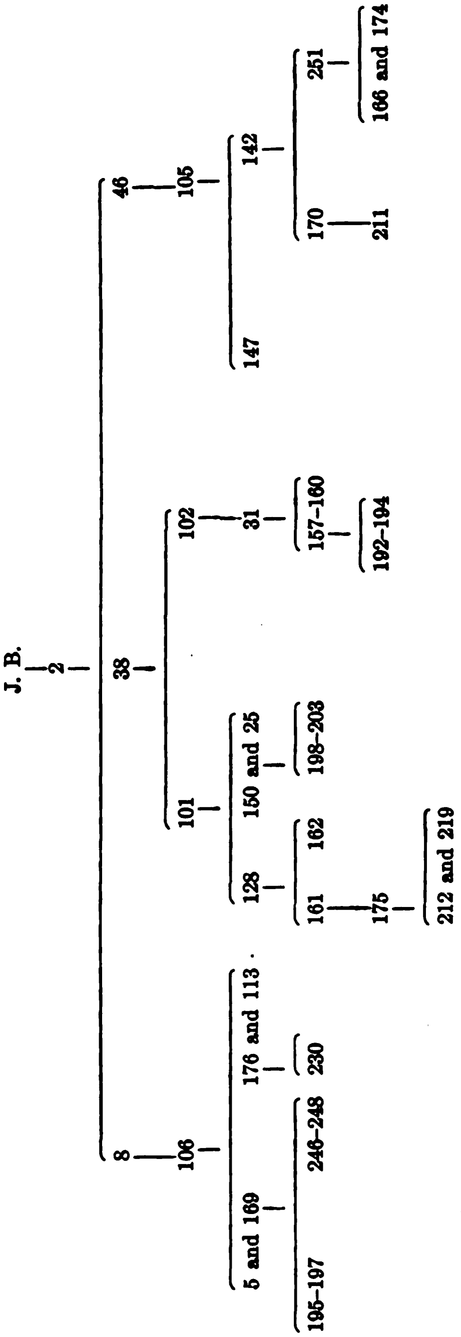
An inspection of the tables shows that the amounts of potassium and calcium are subject to most remarkable variations; rapidly-growing, large tumors showing a marked tendency to a high potassium and low calcium content, whilst the reverse is to be observed in the case of slow-growing tumors. This is so marked that in certain cases of very early tumors potassium only is to be recognized, whilst in the majority of old, necrotic tumors calcium only is present. It will be seen, furthermore, that a steady fall from a high percentage of potassium in the younger to practically none in the older tumors is accompanied by a corresponding increase from no calcium in the younger, to a high percentage of calcium in the older, necrotic tumors (Tables III, VI, VII, VIII).

To indicate clearly the relationship existing between the different groups of tumors, a small family tree containing some of the most important series in which chemical analyses have been carried out, is appended (see Table I). When a "lot" of mice is inoculated it receives an experimental number, and each individual mouse in that "lot" receives a letter and a characteristic mark for future recognition. Every few days each mouse is charted; that is to say, in the series of books provided for that purpose diagrams of the back are prepared, showing the head and the fore and hind legs, and if a tumor is developing a life-size drawing is made, showing its exact location. We are thus enabled to follow the development of the tumor with some accuracy and to form a fair estimate as to the rapidity of its development. At death, the mouse plus tumor, and the tumor alone are weighed, and a portion of the latter is hardened for microscopical examination.

There are four main groups from which the tumors used for analyses have been derived, lots 101, 102, 105, and 106. Lots 101 and 102 have an immediate common ancestor. All four lots have a common ancestor through Lot 2, in the Jensen mouse two generations previously. To facilitate an examination of the figures, the analytical results have been arranged under the four individual groups designated above, dividing the members of the group into the original series and the first, second, third, and fourth generations.

In the tables are given, first, the analytical number of the tumor for purposes of reference, followed by the experimental number and letter of the mouse. In the next column is the origin of the tumor, the immediate ancestor being first given, followed by preceding ancestors until one of the four groups, 101, 102, 105, or 106 is reached. This is followed by the date of inoculation; and the date on which the tumor had reached the size of a buckshot, having a diameter of fully 1½ to 2 mm. The date of death or the date on which the animal was killed is then recorded. This is followed by the number of days which elapsed between the first appearance of the tumor and the date of death and the number of days from inoculation to death, the two different periods being given in order to demonstrate the relationship which obtained in some cases with one and in some cases with the other. This is followed by the weight of the mouse as a whole and the weight of the tumor, and the weight of the tumor material used for analysis in order that an estimate of the relative accuracy of such an analysis may be formed from the amount of materials employed for that purpose. The total weights of sodium, potassium, and calcium are then recorded, followed by the percentage of these elements in the original materials for analysis.

TABLE I.


J. B.
5 and 169
195–197
246–248
176 and 113
212 and 219
150 and 25
198–203
157–160
192–194
166 and 174

In making use of these figures it must be borne in mind that in spite of the greatest precautions observed in analytical work, considerable variations necessarily occur in the amount of water in the materials used for analysis. Consequently it is always advisable to compare the amount of one element with the amount of another rather than the absolute quantities of each present in a given weight of tumor. In addition to these tables others have been prepared supplementing the figures recorded in the general tables, in which the total weights of the three bases, sodium, potassium, and calcium, have been added and the percentage of each determined (Tables III, VI, VII, VIII). Whilst the analytical data in the majority of cases are obtained from single tumors, in one case (Table VII) the results from all tumors falling within given age periods are averaged, a method which affords a more accurate means of estimating the percentage of potassium or calcium, since by this means a considerable error, introduced by the use of small tumors, is counteracted by the more reliable results obtained from the analyses of the larger tumors.

The earliest offshoot from the original Lot 2 was Lot 106, and although the tumors from this lot were not analyzed, the descendants may well be considered first. In the first generation eight analyses are recorded, five of them in the family of 169 and one in Lot 5 (reinoculation), all derived from 106 A, whilst the two remaining tumors are from Lot 176, inoculated from 106 B (see Table II).

In the second generation eight analyses are recorded, three from Group 195, inoculated from 169 C; three from Groups 246, 247, and 248, inoculated at a later date with 169 P, and two of Group 230, derived from 106 through 176 B. Generally speaking, the earliest members of the series show a preponderance of potassium and the later members a preponderance of calcium, and when the immediately related tumors are considered in their respective groups it will be seen, for example, that in the 195 group a steady fall in potassium takes place, whilst in the derivatives of 169 P there is a rise in calcium. In the 230 groups, B, which died first, has a higher potassium and lower calcium content than A. One tumor only is given in the third generation of 106,

TABLE II.

LOT 106.—FIRST, SECOND AND THIRD GENERATIONS.

No.	Exp. No. and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of Tumor in Days.		Weight of mouse.	Weight of tumor.	Wt. of tumor for analysis.	Weight of Na.	Weight of K.	Weight of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
First Generation.																	
13....	169 A	106 A	Jan. 7	Jan 17	Jan. 30	....	13	23	11.7	2.7	2.0	0.0061	0.004	trace	0.30	0.2	....
15....	169 E	106 A	Jan. 7	Jan. 17	Feb. 1	....	15	25	20.5	7.6	5.0	0.0195	0.0055	trace	0.39	0.111	....
16....	5	106 A	Jan. 7	Jan. 22	Feb. 3	....	12	27	29.0	9.0	6.47	0.0303	0.0051	trace	0.46	0.079	....
18....	169 F	106 A	Jan. 7	Jan. 17	Feb. 6	....	20	30	12.5	3.5	3.1	0.0122	0.0041	trace	0.39	0.132	....
25....	169 G	106 A	Jan. 7	Jan. 17	Feb. 20	....	34	44	18.5	4.5	4.22	0.0165	0.0032	0.0016	0.39	0.075	0.039
30....	176 A	106 B	Feb. 1	Feb. 11	Mar. 13	....	30	40	22.0	7.8	6.9	0.0266	0.0076	0.0022	0.38	0.111	0.033
49....	169 O	106 A	Jan. 7	Jan. 28	....	Apr. 14	76	97	29.0	6.32	3.4	0.0156	0.001	abs.	0.46	0.029	....
51....	176 B	106 B	Feb. 1	Feb. 15	....	Apr. 14	58	72	33.6	7.9	4.3	0.016	0.0035	0.0018	0.37	0.082	0.042
Second Generation.																	
48....	195 D	169 C–106	Feb. 24	Mar. 15	....	Apr. 14	30	49	23.5	7.0	4.1	0.0193	0.0035	abs.	0.47	0.087	....
56....	195 F	169 C–106	Feb. 24	Mar. 15	Apr. 24	....	40	59	20.0	5.0	4.3	0.0241	0.0012	0.0017	0.56	1.028	0.037
59....	195 G	169 C–106	Feb. 24	Mar. 30	May 9	....	41	75	....	....	10.2	0.0421	0.0025	0.003	0.41	0.025	0.029
68....	230 B	176 B–106	Apr. 14	Apr. 24	....	May 23	29	39	23.8	7.6	4.9	0.02	*0.0009	0.0012	0.41	*0.019	*0.024
75....	247 C	169 P–106	Mar. 22	Apr. 20	June 5	....	36	65	....	5.9	5.0	0.0187	0.0014	0.0009	0.37	0.028	0.018
83....	246 B	169 P–106	Mar. 22	Apr. 14	....	June 5	52	75	31.6	10.5	7.1	0.027	0.0029	0.0046	0.37	0.04	0.065
88....	248 D	169 P–106	Mar. 22	May 2	June 8	....	37	78	....	....	2.8	0.0097	abs.	0.005	0.35	....	0.18
92....	230 A	176 B–106	Apr. 14	Apr. 20	June 17	....	58	64	....	....	6.8	0.024	0.0007	0.0075	0.35	0.01	0.11
Third Generation.																	
65....	232 A	195 D– 169 C–106	Apr. 14	Apr. 22	....	May 19	27	35	27.0	6.7	4.6	0.0206	0.0018	0.0009	0.44	0.038	0.019

*Slight loss of K.

and it will be seen to correspond fairly well with the members of the series from which it is derived.

Lot 169 is dealt with independently in Table III. The total content of the three bases, sodium, potassium, and calcium, is determined, and the percentage of potassium of this total is shown for each member. It will be seen that a steady fall takes place from 40 per cent. in the first of the series to 6 per cent. in the last. The most marked characteristic of this group is the very high percentage of potassium and the practical absence of calcium. One tumor only, 169 G, shows a weighable amount of calcium. In noting the fact that 169 O, which died long after 169 G, shows no calcium, whilst 169 G contains a certain amount, it must be remarked that 169 O was killed whilst G died. In comparing the two tumors in the chart-book, 169 G is seen to have grown rapidly at first and to have become necrotic later, whilst 169 O grew very slowly and steadily at first, but extremely rapidly in the course of the twenty days immediately preceding the date on which it was killed.

TABLE III.

GROUP 169.

Number.	Experiment number and letter.	AGE OF TUMOR.		Weight of Na.	Weight of K.	Weight of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.	REMARKS.
		1st app. to death.	Inoc. to death.							
13	169 A	13	23	0.0061	0.0040	trace	0.0101	40	....	
15	169 E	15	25	0.0195	0.0055	....	0.0250	22	....	
18	169 F	20	30	0.0122	0.0041	trace	0.0163	25	....	
25	169 G	34	44	0.0165	0.0032	0.0016	0.0213	15	7.5	Grew rapidly at first. became necrotic later.
49	169 O	76	97	0.0156	0.0010	trace	0.0166	6	....	Grew slowly at first, but extremely rapidly in last 20 days, and was killed whilst still developing.

We now pass on to the derivatives of Lot 101 in which the largest number of analyses are to be found (Tables IV and V). In the first generation four analyses only were completed, and unfortunately in one case the calcium was lost through accident.

From a casual inspection of the table there would appear to be absolutely no relationship between the potassium and calcium con-

TABLE IV.

LOT 101.—FIRST AND SECOND GENERATIONS.

No.	Exp. no. and letter.	Origin.	Date of inoc.	Date. of 1st app.	Died.	Killed.	Age of tumor.		Weight of mouse.	Weight of tumor.	Wt. of tumor for analysis.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
							First Generation.										
11....	25	101	Dec. 5	Jan. 3	Jan. 28	....	25	44	18.7	2.8	2.4	0.0086	0.0038	0.0011	0.360	0.161	0.046
22....	119	101	Dec. 5	Dec. 31	Feb. 17	....	48	74	21.5	7.0	6.8	0.0133	abs.	0.0022	0.19	....	0.032
26....	152	101	Dec. 5	Jan. 16	Feb. 23	....	38	80	28.5	6.0	4.2	0.0113	0.0027	trace	0.126	0.065	....
38....	150 C	101	Dec. 5	Feb. 14	Mar. 30	....	44	115	28.6	8.2	5.2	0.0235	0.0030	0.0036	0.44	0.057	0.069
							Second Generation.										
19....	161 O	128 -101	Dec. 21	Jan. 3	Feb. 9	....	37	50	......	7.0	4.8	0.0158	0.0039	0.0016	0.33	0.082	0.033
23....	162 C	128 -101	Dec. 21	Jan. 10	Feb. 17	....	38	58	......	......	4.7	0.0184	0.0017	0.0007	0.39	0.036	0.014
37....	199 G	150 B-101	Feb. 28	Mar. 9	Mar. 28	....	19	28	29.0	10.5	8.8	0.0408	0.0087	0.0016	0.46	0.099	0.018
39....	198 G	150 B-101	Feb. 28	Mar. 15	Apr. 1	....	17	32	17.0	4.0	3.0	0.0130	0.0058	0.0010	0.43	0.193	0.033
41....	198 F	150 B-101	Feb. 28	Mar. 15	Apr. 7	....	23	38	24.0	8.0	6.0	0.0214	0.0060	0.0014	0.36	0.100	0.027
42....	201 G	150 B-101	Feb. 28	Mar. 9	....	Apr. 7	29	38	18.5	5.5	3.4	0.0163	0.0033	0.0009	0.47	0.096	0.026
43....	198 E	150 B-101	Feb. 28	Mar. 6	....	Apr. 7	32	38	18.5	4.0	2.2	0.0105	0.0032	0.0011	0.46	0.142	0.049
44....	201 H	150 B-101	Feb. 28	Mar. 12	....	Apr. 8	27	39	16.1	4.9	4.8	0.0178	0.0027	0.0013	0.37	0.059	0.027
52....	199 D	150 B-101	Feb. 28	Mar. 15	Apr. 20	....	36	51	22.3	7.5	6.4	0.0308	0.0018	0.0011	0.49	0.028	0.017
53....	200 K	150 B-101	Feb. 28	Mar. 12	Apr. 20	....	39	51	......	4.5	4.2	0.0196	0.0004	0.0010	0.47	0.010	0.024
55....	199 E	150 B-101	Feb. 28	Mar. 9	Apr. 17	....	39	48	15.5	5.0	2.5	0.0189	0.0017	0.0013	0.75	0.067	0.052
57....	201 K	150 B-101	Feb. 28	Mar. 10	Apr. 27	....	48	58	......	......	4.8	0.0175	0.0024	0.0013	0.36	0.050	0.027
74....	203 A	150 B-101	Feb. 28	Apr. 17	June 3	....	47	95	......	......	4.1	0.0164	0.0008	0.0052	0.40	0.019	0.125
99....	202 D	150 B-101	Feb. 28	May 2	June 23	....	52	115	16.6	3.7	2.3	0.0123	abs.	0.0077	0.53	....	0.336

tent and the age of the tumor. This group, however, affords an interesting illustration of the possibility of rearranging the series according to the periods of development of the tumor from its first appearance to the death of the animal, instead of the full period from the date of inoculation to death. As a general rule these two periods bear a fairly constant relationship to one another, but in this particular group that is in no sense the case, the second, according to the general form of arrangement, becoming the last if we adopt the other method.

From the small supplementary Table VI it will be seen, summing the total bases and determining their respective percentages, that, grouping the tumors according to their absolute age, potassium falls steadily from 32 per cent to nil, whilst calcium rises from 9 to 15.

Table IV also includes the second generation of Lot 101, consisting of two tumors, 161 O and 162 C, derived from Lot 128, and twelve tumors belonging to Groups 198 to 203, all of one family, obtained by inoculation from 150 B on February 28. This group, derived from 150 B, is one of the most interesting in the entire series, fifty mice inoculated having yielded upwards of twenty large, fairly rapidly growing tumors. Whilst the twelve analyses recorded show a tendency to a steady decrease in potassium and a gradual rise in calcium, reaching very considerable proportions in the last two members of the series, there is at first sight a lack of strict uniformity in the results. When, however, it is remembered that 201 G and 198 E were killed, which appreciably interferes with the results, and that certain of the tumors were rather small for analytical purposes, such discrepancies may readily be accounted for.

In order to reduce the experimental error to a minimum, we have grouped all the mice dying within given periods, adding together the total weight of their tumors, the total sodium, potassium, and calcium content, and determining the potassium and calcium percentage of this total. In Table VII the results are recorded, and show a most remarkable steady fall from 23.7 per cent potassium in the twenty to thirty-five day period to nothing in the one hundred and ten to one hundred and thirty-five day

TABLE V.

Lot 101. — Third and Fourth Generations.

Number.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor. 1st app. to death.	Age of tumor. Inoc. to death.	Weight of mouse.	Weight of tumor.	Weight of tumor for analysis.	Weight of Na.	Weight of K.	Weight of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
								Third Generation.									
36....	254 B	161 M-128-101	Jan. 31	Mar. 2	....	Mar. 28	26	56	28.5	5.6	4.6	0.0186	0.0027	0.0016	0.40	0.059	0.035
40....	175 D	161 M-128-101	Jan. 31	Feb. 17	Apr. 6	....	48	65	15.3	2.9	2.4	0.0090	0.0030	0.0030	0.37	0.125	0.025
78....	228 A	201 G-150 B-101	Apr. 7	Apr. 28	June 5	....	38	59	.....	....	7.6	0.0328	0.0013	trace	0.43	0.016	......
79....	206 H	161 S-128-101	Mar. 3	Apr. 6	June 5	....	60	94	29.8	6.4	3.4	0.0148	0.0018	0.0011	0.43	0.053	0.032
89....	225 A	198 B-150 B-101	Apr. 7	Apr. 13	June 13	....	61	67	.....	2.0	2.0	0.0121	abs.	0.0011	0.62	......	0.056
93....	237 B	199 F-150 B-101	Apr. 19	May 17	June 20	....	34	62	.....	....	6.5	0.0272	0.0022	0.0027	0.41	0.034	0.041
98....	304 D	200 A-150 B-101	Apr. 26	May 23	June 23	....	31	58	19.2	4.1	3.4	0.0159	0.0014	0.0008	0.46	0.042	0.023
								Fourth Generation.									
58....	213 C	175-161-128-101	Mar. 28	Apr. 20	May 8	....	18	41	.....	....	2.9	0.0138	0.0019	0.0007	0.47	0.064	0.024
62....	215 A	175 C-161 M-128-101	Mar. 28	Apr. 13	May 15	....	32	48	.....	....	2.9	0.0111	0.0005	0.0008	0.38	0.016	0.027
67....	216 B	175 C-161 M-128-101	Mar. 28	Apr. 22	....	May 23	31	56	26.0	7.5	4.6	0.0214	abs.	0.0017	0.46	......	0.036
72....	110	175 C-161 M-128-101	Mar. 28	....	June 2	....	....	66	.....	....	6.7	0.0078	abs.	0.0026	0.11	......	0.038
81....	213 A	175 C-161 M-128-101	Mar. 28	Apr. 17	June 6	....	50	70	.....	....	4.4	0.0159	abs.	0.0030	0.36	......	0.068
87....	218 B	175 C-161 M-128-101	Mar. 28	Apr. 27	June 9	....	43	73	.....	....	5.0	0.0151	abs.	0.0025	0.30	......	0.049
91....	116	175 C-161 M-128-101	Mar. 28	....	June 14	....	....	78	.....	....	6.4	0.0214	abs.	0.0082	0.33	......	0.127

period, whilst the calcium content rises steadily from 4.2 per cent in the former to 38.5 in the latter periods.

Table V includes the third and fourth generation of Lot 101. In the third generation there is very little basis for comparison of the results. In the first two tumors the ratio of potassium over calcium falls slightly with increase in age. Of the four tumors derived from 150 B through the 198 to 203 group, it will be seen that the three which take from thirty to forty days for development from the date of first appearance show potassium and about equal amounts of calcium, whilst the remaining member of the group, 225 A, in which the period of development was sixty-one days, shows no potassium and the highest amount of calcium. In the fourth generation of 101, also recorded on this sheet, is the most remarkable series of individual tumors thus far encountered. All seven members of this generation were derived directly from 175 C and are, therefore, more strictly comparable than is

TABLE VI.

First Generation of 101.

No.	Experiment number.	Age inoculated to death.	Weight Na.	Weight K.	Weight Ca.	Total bases.	K per cent.	Ca per cent.
11	25	25	0.0086	0.0038	0.0011	0.0135	28.1	8.1
26	152	38	0.0113	0.0027	Lost	0.0140	20.0	Lost
38	150	44	0.0235	0.0030	0.0036	0.0301	10.0	12.0
22	119	48	0.0133	abs.	0.0022	0.0155	......	15.0

TABLE VII.

Experiment Number and Letter.	Age of tumors in day periods.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.	Ratio of K to Ca.
199 G–198 G..........	20–35	0.0436	0.0145	0.0026	0.0607	23.7	4.2	5.6
198 F–201 G–198 E–201 H..................	35–50	0.0660	0.0152	0.0047	0.0859	17.6	5.4	3.2
199 E–201 K........ 199 D–200 K........	50–65	0.0868	0.0063	0.0047	0.0978	6.4	4.7	1.4
None.................	65–80	....	....	....	....	......	......	......
203 A.................	80–95	0.0164	0.0008	0.0052	0.0224	3.5	23.0	0.15
None.................	95–110	....	....	....	....	......	......	......
202 D.................	110–125	0.0123	nil	0.0077	0.0200	nil	38.5	......

generally the case. A steady fall in the potassium content is associated with an equally steady rise in the calcium content. This remarkable agreement is emphasized in the special Table VIII, in which the total bases are added together and the potassium and calcium percentage determined. It should be noted that 216 B was subjected to a treatment previous to analysis whereby a certain amount of potassium may have been lost. This tumor, although falling into the series, should therefore be excluded from any strict comparison. One member of the fifth generation of 101, No. 327 B, showed at the age of forty-seven days .117 per cent of calcium and no potassium.

Table IX gives the results of analyses carried out on the tumors derived from 102. Only six mice have been used for this purpose in the three generations, and it will be seen at a glance that the same general relationship obtains as in previous cases.

TABLE VIII.

No.	Exp. no. and letter.	Age of tumor.		Weight of Na.	Weight of K.	Weight of Ca.	Total of bases.	Per cent of K.	Per cent of Ca.
		1st app. to death.	Inoc. to death.						
58	213 C	18	41	0.0138	0.0019	0.0007	0.0164	12.0	4.3
62	215 A	32	48	0.0111	0.0005	0.0008	0.0124	4.0	6.6
67	216 B	31	56	0.0214	trace	0.0017	0.0231	*trace	7.4
81	213 A	50	70	0.0159	trace	0.0030	0.0189	....	16.0
87	218 B	43	73	0.0151	trace	0.0025	0.0176	....	14.0
91	116		78	0.0214	trace	0.0082	0.0296	....	27.4

*Lost.

Lot 105 and its derivatives constituting the fourth important group are considered in Table X. Whilst five analyses of actual members of Lot 105 were carried out, two only have been included, the other three being amongst the first work of this nature carried out in the laboratory, and a certain amount of question existing as to their accuracy. It may, however, be said that in no case was potassium found to be present, although the ages of the tumors ranged from 42 to 107 days. Calcium was present in considerable quantities in all five tumors. In the first generation two tumors analyzed show no potassium and a fair amount of calcium. In the second generation one small tumor shows potassium and a second, at 67 days, no potassium and a large content of calcium. In the third generation are five tumors, three of them derived from 251 E. 251 E was utilized originally for a series of experiments in which the virulence of the normal materials admixed with salt solution at normal temperatures, at 40° and 45°, was compared. In this case the materials incubated fifteen minutes at 40° gave a yield of a larger number of more rapidly growing tumors than was obtained from the use of the normal material.

This work will be the subject of another paper from this laboratory, and will not therefore be dealt with at this stage, but it should simply be noted that the tumors in question made their appearance only twelve days after inoculation and grew with extreme rapidity, and when examined macroscopically appeared to be necrotic, especially the earliest members of the series. This may account for the practical absence of potassium and the high calcium content of this group.

In the fourth generation of 105 are seven tumors, five of them derived from 174 G and one each from 174 B and 174 F. When this series is grouped collectively in definite periods, the same type of relationship is found to obtain as in previous cases, the only marked discrepancy being the absence of potassium in 322 C and 329 A, preceding 233 A in actual age, but it must be noted that 233 A grew rapidly at the last stage, whilst 322 C and 329 A after a rapid early development were practically stationary, were commencing to ulcerate and already becoming distinctly necrotic.

To summarize the above analytical results, it may be said that, both as a whole and also in individual groups, the most rapidly developing tumors which reach large dimensions in the course of a short time show a high potassium content and practically no calcium. The pathological examination of sections of such tumors, for example, 169 E and 5 in Table I and 199 G, 198 G and 198 F in Table IV, shows rapidly growing cancer epithelium and relatively small indications of necrosis. These tumors contained from .05 to .09 gram of potassium, an amount which is exceptional when the extremely small size of the tumors is considered. In contrast to the rapidly growing tumors, showing a high per

TABLE IX.

LOT 102 AND DERIVATIVES.

Number.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	AGE OF TUMOR.		Weight of mouse.	Weight of tumor.	Weight of tumor for analysis.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
							First Generation.										
14....	115 C	102	Nov. 12	Nov. 30	Jan. 31	....	62	80	25.8	6.6	4.9	0.0161	0.0053	trace	0.33	0.109	......
27....	129 A	102	Nov. 12	Nov. 30	....	Feb. 28	90	108	29.5	10.0	3.2	0.0116	0.0020	0.0030	0.35	0.061	0.092
							Second Generation.										
12....	160 N	31–102	Dec. 23	Jan. 3	Jan. 28	....	25	36	11.8	2.9	2.5	0.0082	0.0034	0.0012	0.33	0.136	0.050
20....	157 A	31–102	Dec. 23	Jan. 12	Feb. 15	....	34	54	....	4.7	3.6	0.0138	0.0016	0.0022	0.38	0.044	0.060
21....	157 B	31–102	Dec. 23	Jan. 20	Feb. 16	....	27	55	12.5	3.0	2.8	0.0117	0.0019	0.0018	0.41	0.067	0.063
							Third Generation.										
35....	192 F	157–31–102	Feb. 22	Mar. 10	Mar. 25	....	15	31	20.0	2.5	2.0	0.0097	0.0015	0.0014	0.48	0.076	0.070

cent of potassium, are the slow-growing tumors, many of them over one hundred days old, which generally contain over .1 per cent of the calcium and no potassium. Such for example, are 203 A and 202 D the two oldest tumors in the second generation of Lot 101, Lot 116, the oldest tumor in the fourth generation of 101, and several tumors amongst the 105 derivatives. Such tumors are not necessarily entirely necrotic. They generally contain a relatively large amount of connective tissue, but this question is one which will be the subject of further investigation in this laboratory.

A further consideration of the tables in detail shows that with tumors of relatively the same size, the age of the tumor, whether estimated on the absolute basis or simply from the time it makes its first appearance, generally bears a certain ratio to the potassium and calcium content, or the ratio of $\frac{K}{Ca}$. Thus, if the ratios of $\frac{K}{Ca}$ are taken in such a group as 198 and 203, for example, in which both elements are present in all tumors with the exception of the last, it is found that $\frac{K}{Ca}$ falls gradually in value from 5.7 and 5.8 at the commencement to .15 and nil at the end of the series. This fact is most clearly illustrated in Table VII, where the ratio for the first period is 5.6, for the second period 3.2, for the third period 1.4, for the fourth period .15, and for the fifth period nothing.

The admixture of small and large tumors is misleading, large tumors having a tendency to show a higher content of potassium than is to be expected at their period of development, small tumors showing a larger content of calcium. If, however, the relative proportions of these elements are functions of the rapidity of growth, this discrepancy will readily be explained when one realizes that although two tumors may, through toxic or other influences, cause the death of mice in the same period of time, if one is twice as large as the other, its rate of development has been considerably higher. In the few cases in which discrepancies occur, a reference to the chart-books generally affords an explanation. It happened, for example, that an old tumor showed a considerable amount of potassium. In such a case it was generally observed that the tumor in question had developed slowly at first but very rapidly in the last stages. On the other hand, when a

TABLE X.

Lot 105 and Derivatives.

No.	Experiment number and letter.	Origin.	Date of inoc.	Date of 1st app.	Died.	Killed.	Age of tumor.		Wt. of mouse.	Wt. of tumor.	Wt. of tumor for analysis.	Wt. of Na.	Wt. of K.	Wt. of Ca.	Per cent of Na.	Per cent of K.	Per cent of Ca.
							1st app. to death.	Inoc. to death.									
6	105	......	Oct. 10	Oct. 28	Dec. 1	......	34	52	14.7	7.5	5.2	0.0154	abs.	0.0017	0.29	......	0.032
10	105 blk.	......	Oct. 10	Oct. 28	Jan. 25	......	89	107	17.7	6.0	3.5	0.0134	abs.	0.0030	0.37	......	0.084
First Generation.																	
17	142-x=ray	105	Nov. 19	Nov. 26	Feb. 6	......	72	79	20.7	4.2	2.8	0.0135	abs.	0.0017	0.48	......	0.060
24	147 C	105	Nov. 30	Jan. 30	Feb. 17	......	18	79	22.0	3.0	2.8	0.0104	abs.	0.0027	0.37	......	0.096
Second Generation.																	
31	149 D	142–105	Jan. 5	Jan. 16	Mar. 13	......	56	67	23.0	5.9	5.0	0.0130	abs.	0.0058	0.25	......	0.115
Third Generation.																	
50	174 F	251 E–142–105	Mar. 17	Mar. 27	......	Apr. 14	18	28	26.1	4.1	2.3	0.0097	abs.	0.0013	0.42	......	0.056
61	174 G	251 E–142–105	Mar. 17	Apr. 1	May 16	......	45	60	......	....	2.9	0.0131	0.0016	0.0090	0.45	0.054	0.030
73	211 C	170 F–142–105	Mar. 15	......	June 3	......		80	......	....	8.1	0.0262	abs.	0.0059	0.32	......	0.072
76	166 A	251 E–142–105	Mar 17	Apr. 10	June 5	......	56	80	......	....	0.8	0.0026	abs.	0.0013	0.33	......	0.168
97	234 F	41 B–147–105	Apr. 19	May 17	June 22	......	36	64	15.1	1.3	1.3	0.0037	abs.	0.0033	0.28	......	0.238
Fourth Generation.																	
70	321 A	174 G–251 E–142–105	May 5	May 17	May 31	......	14	26	......	....	4.0	0.0229	0.0005	trace	0.57	0.013	......
77	321 D	174 G–251 E–142–105	May 5	May 20	June 5	......	16	31	......	....	3.7	0.0166	0.0029	0.0013	0.44	0.075	0.034
82	322 A	174 G–251 E–142–105	May 5	May 20	June 6	......	17	32	......	....	4.9	0.0215	0.0020	0.0008	0.43	0.041	0.016
90	324 A	174 G–251 E–142–105	May 5	May 23	June 15	......	23	41	......	....	5.4	0.0241	0.0012	0.0014	0.44	0.022	0.025
95	233 A	174 F–251 E–142–105	Apr. 14	Apr. 24	June 21	......	58	68	24.5	7.0	6.3	0.0257	0.0015	0.0033	0.41	0.023	0.052
96	322 C	174 G–251 E–142–105	May 5	May 27	June 22	......	26	48	17.5	5.8	5.3	0.0220	abs.	0.0019	0.41	......	0.035
101	329 A	174 B–251 E–142–105	May 16	May 29	June 28	......	30	43	19.5	6.0	5.2	0.0213	abs.	0.0008	0.35	......	0.015

large quantity of calcium is present in a young tumor, it is generally observed that the tumor in question grew rapidly at first, then remained stationary and became necrotic previous to the death of the mouse.

In contrasting the descendants of the three main groups, 106, 101, and 105, it appears at first sight that the derivatives of 106, especially the 169 group, show a preponderance of potassium; that is to say, that at a given age and given size, and under otherwise comparable conditions, the members of this group appear to contain more potassium than do those of Group 101 and 105. Group 105, at least in the early generations, shows in the same sense a high proportion of calcium, whilst Group 101, especially through the strain of 150, shows a more even balance of these two elements than is to be found in either of the other groups.

The series of mice from 198 to 203, which, as was stated above, has yielded a higher proportion of large, rapidly growing tumors than any other series so far investigated, is most remarkable for the fact that with one exception every tumor of this series contains both potassium and calcium in appreciable quantities, the proportion of potassium to calcium obtained by adding together the total content of twelve tumors being 3 : 2.

In considering the results of transplantation from young tumors and old tumors, we made the observation early in the work, that even rapidly growing young tumors are not suitable for this purpose; rapidly growing, partially necrotic, older tumors giving better results.

One of the most remarkable examples of a high calcium content in a young tumor is 174 F, which was killed twenty-eight days after inoculation. This tumor appeared, however, from the very first to be necrotic, and on reinoculation produced similar, although less marked results in the next generation.

In the course of these experiments six additional tumors were dried and extracted with chloroform in order to remove fatty bodies, and it is interesting to note that the potassium constituents were to a considerable extent dissolved by the extracting agent, whilst the calcium remained entirely in the residual portion.

The potassium and calcium content in its relation to virulence.—Two series of experiments were carried out. In one series twelve tumors were used both for purposes of inoculation and also for chemical analysis. In the second series of experiments tumor materials from the same source were mixed, on the one hand with a potassium-chloride and on the other hand with a calcium-chloride solution, isotonic with normal salt solution, and used subsequently for injection.

In the first series of experiments 198 F, 201 G, 198 E, and 198 B were employed on April 7 for inoculation. 198 F and 201 G, having a slightly higher proportion of potassium than 198 E, showed a higher proportion of tumors. This experiment can scarcely be considered conclusive on account of the smaller number of mice, five only being employed in each series. On April 14 four mice, 195 D, 169 O, 174 F, and 176 B were used for a similar purpose; whilst the chemical analyses showed very considerable variations, the yield of tumors in each case was fairly comparable. Further experiments carried out on May 19, on 232 A, with a larger proportion of potassium than 200 L, gave four tumors as against one in 200 L, eight mice being employed in each case. These experiments are in no sense conclusive; and it will be necessary to do a considerable amount of experimental work in this direction, on account of great variations shown in the relative virulence of different tumors.

Experiments to determine the direct effects of isotonic solutions of potassium and calcium upon the development of tumors have been undertaken and are now under way. All that can be said at the present time is that the mice inoculated and fed with potassium-holding materials appear to be more susceptible than those inoculated and fed with calcium material. No attempt has been made at present to draw conclusions based on the ionic theory, but it is interesting to note that the figures in the tables showing the ratio of potassium and calcium apply also to the ions of these elements, their relative atomic weights, 39 and 40, producing comparatively small change.

It is not our purpose to speculate at present regarding the significance of the proportions of these elements in the tumors, and we are not disposed to attribute any direct influence to the potassium as such, but consider the potassium rather as a necessary element in active growth, and the calcium a result of necrosis.

It must, however, be borne in mind that calcium has been shown to exert an inhibiting influence upon potassium in a large variety of the physiological effects of this element.

Considering the great length of time through which these tumors have been transplanted, it will be realized that an immense number of subdivisions of the original cell must have taken place. It is possible that work similar to the above, carried out for a considerable period, may show evidence of an actual aging process and loss of the dividing-energy. We have already been led to suspect the existence of a form of periodicity in the virulence of the mouse tumors. There is a certain amount of evidence in the tables given above that the same may be said to exist as regards chemical composition. It is proposed to make careful observations regarding any indications of a rejuvenation process in its relationship to the varying chemical composition of the tumor series.

SUMMARY.

It has been shown that rapidly growing, large tumors in mice contain a high percentage of potassium and little or no calcium; also that old, slowly growing, relatively necrotic tumors contain large amounts of calcium and little or no potassium.

It has been observed that in groups of comparable mice the potassium and calcium content is a function of the age and rapidity of development of the tumor, discrepancies observed being frequently attributable to more rapid growth or degenerative processes already recorded in the diagram books.

Certain groups appear to possess at given ages relatively larger proportions of potassium and others relatively larger proportions of calcium; but the largest yield of tumors and the most rapid growth as represented by the unit of tumor produced per unit of mice in the unit of time, is apparently associated with an equilibrium in which both elements are present, the ratio averaging from 2 : 1 to 3 : 2 in favor of potassium.

Some Artefacts in Mouse Carcinoma.*

By G. N. Calkins and G. H. A. Clowes.

The present paper deals with certain cell inclusions in mouse carcinoma, inclusions due to technical errors, but offering some evidence of an interesting type of intracellular necrosis.

The history of cell inclusions in carcinoma clearly shows that products of necrosis have been occasionally mistaken for parasites of one kind or another, and the possibility — especially after fixation with any mixture containing salts of mercury — of similarly mistaking artefacts is apparent to all laboratory workers.

The difficulty in removing precipitates from Zenker-fixed material is generally recognized, and treatment in bulk with iodized alcohol (often for weeks), is advised in most laboratory manuals, and for large pieces, subsequent treatment of paraffin sections is recommended. Rawitz's *Leitfaden für histologische Untersuchungen* advises treatment of pieces fixed in Zenker with "70 per cent alcohol with enough tincture of iodine added to give it a port wine color, the iodine and alcohol to be changed until the color no longer disappears" (1895, p. 17). Mercier[1] recommends ten to fifteen days at least in iodized 90 per cent alcohol and advises the use of clear tincture of iodine or strong concentrated iodized alcohol.

The inclusions that we have to describe are not affected perceptibly by such treatment. The usual treatment of material in this laboratory is the same as that recommended in the majority of laboratory manuals, and the usual "mercury" deposits are frequently seen and recognized as such. In the series of mouse experiments with which the State Cancer Laboratory has been occupied for more than a year, such inclusions have been seen in the cancer tissues and passed over without much thought. In one case, however, the tumor when partially grown was stirred up thoroughly with a needle and then left for further developments. After some days it was observed that the tumor had grown smaller and the mouse showed signs of spontaneous recovery. The tumor was then removed and hardened in Zenker's fluid, treated as

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* Received for publication July 20, 1905.

[1] *Zeit. für w. Mik.*, 1894, 11, p. 471.

usual with iodized alcohol, embedded in paraffin, sectioned and stained according to the usual methods. The most wonderful variety of crystals and deposits, both intra and intercellular, were found in these sections, some comparatively large, others exquisitely minute.

The most common type of crystalline deposit in this tumor is comparatively small, dense, and nearly homogeneous, and with clear evidence of radiate structure usually with an eccentric focus. In other forms the radii are more marked, clearly defined, and the entire structure is fan-shaped (Plate I, Figs. 1 and 4). In still other cases this fan structure is elaborated in much greater detail and many of the radii are carried out beyond the periphery as elongate, filamentous processes (Plate II, Fig. 1). The ends of these processes bear local, cylindrical or sausage-shaped thickenings which give to the structure a striking resemblance to a fungus-growth.

In other cases of intercellular inclusions the crystallization does not result in uniform, definite masses, but in filaments without the dense basal portion. Such structures may start from the periphery of a hypertrophied cell, in which irregular masses of the deposit and the degenerated nucleus alone remain; while at the ends of the filaments again, are the enlarged, clublike, cylindrical masses (Plate II, Fig. 2).

In still other cases the type of the crystallization differs from both of the above, resembling more than anything else the plan of structure of hoar frost on glass. Minute crystalline masses are attached end to end and a running crystalline structure results which may extend over an area of at least one-half a square millimeter.

The intracellular and intranuclear inclusions are even more remarkable than the intercellular. For convenience they may be distinguished as the "cytoplasmic" and the "nuclear" forms. The cytoplasmic forms are always in vacuoles which vary in size from minute vesicles to great intracellular spaces considerably larger than the nucleus. These small vesicles are seen in the vicinity of the nucleus and usually in the minute depression formed by the cresent-shaped nucleus (Plate II, Figs. 6–10). Within the vacuoles are many curious, separate, filiform structures with characteristic spherical swellings at the ends (Plate I, Figs. 5 and 6; Plate II, Figs. 9 and 10). These filaments are usually single and separate, but in some cases they form a botryoidal group of characteristic arrangement (Plate II, Fig. 8). Occasionally there is a central mass with irregular radiating branches (Plate II, Fig. 13), while in a few cases the radiating structure, central mass, and knobbed radii furnish an intracellular parallel to the larger intercellular fan-shaped structures (Plate II, Fig. 12).

Even more remarkable are the intranuclear filaments and knobs. Inside the nucleus there are minute masses similar in size and shape to the swollen ends of the radiating filaments (Plate II, Fig. 5); or larger, swollen masses with irregular protuberances of variable length (Plate II, Figs. 4 and 5), or finally, they may be long filaments with knobs on the ends similar to the knobbed filaments of the cytoplasmic forms (Plate II, Fig. 3; Plate I, Fig. 3).

Attempts to stain these various structures are not successful; in some cases the structures have a faint homogeneous color which cannot be interpreted as a true staining reaction. The filaments and knobs are never stained even as much as this, although blackened by treatment with ammonium sulphide. Usually they present the same glassy or glairy appearance that characterizes fat under the microscope, and this appearance is more definite in the terminal swellings than in the substance of the filaments or the rays of the fan structure.

Unfortunately, on account of the limited amount of material at our disposal, it was impossible to make a thorough chemical investigation of these products. From micro-chemical tests, however, it was found:

That a prolonged treatment with cold Lugol's solution removes all the bodies referred to above, a slight, amorphous residue being left within a few of the vacuoles.

That a short treatment with Lugol's solution leads to partial removal of the crystals, especially the large, extracellular, fan-shaped variety.

That treatment of the sections with dilute sulphuretted hydrogen or ammonium sulphide leads to a distinct blackening, the intensity of which is dependent upon the length of time that the sections are exposed to the reagent and the concentration of the latter. Sections so treated, when subsequently exposed to Lugol's solution at 50°, are entirely cleared in the course of half an hour.

These tests warrant the conclusion that the semi-crystalline compounds consist, at least in part, of mercury salts derived from the hardening agent. It should, however, be noted that whilst the large fan-shaped bodies blacken intensely under the influence of ammonium sulphide, the smaller intracellular and intranuclear bodies are blackened to a less marked extent and the fine filaments to which knobs are attached (Plate II, Figs. 6–10), are apparently influenced very slightly, if at all.

The solubility of the various types of inclusions was tested in a series of organic solvents. Alcohol exerts very little influence in the cold, but at 50° for half an hour sections are entirely cleared of all inclusions, merely a slight residue being observed in some of the vacuoles. Ether, acetone, and admixtures of alcohol, ether, and acetone exert a similar, though possibly less marked, solvent action than does alcohol alone. Petroleum-ether and xylol produce very little effct, even at 50°. Chloroform apparently exerts more action than the latter and less than the former group of reagents. These results, when taken with the fact that salts of mercury are far more soluble at high temperatures in organic solvents than at normal temperatures, further justify the conclusion that the structures described above consist, at least in part, of salts of mercury.

To summarize the above results of chemical tests it may be said that mercury is unquestionably present in larger or smaller quantities in all the inclusions, both intra and intercellular. From the behavior of the crystals, especially the intracellular and intranuclear varieties, toward ammonium sulphide and other reagents it appears possible that the mercury salt is either deposited upon some product of necrosis of a fatty nature, or intimately associated with the latter in the form of a chemical compound. It has been found impossible to reproduce such forms from normal tissue or by means of a hardening agent admixed with various known products of decomposition. If any peculiar products of necrosis are associated with the salts of mercury in the inclusions referred to, the readiness with which they are removed by hot organic solvents and their behavior toward Lugol's solution and other reagents, as well as their lack of any characteristic microchemical reactions would lead us to suppose that they are of a fatty nature. In any case, it must be said that the figures observed resemble very closely the various forms assumed by fatty acids and fatty acid compounds when admixed with impurities.

While these deposits are to be interpreted as artefacts, the fact must not be overlooked that something of an unusual nature is present in these cancer cells and tissues, upon which the salts of the fixing agent work in forming the deposits of various kinds. The morphological evidence supports the view that necrosis of a marked type is in progress in these cancer cells; a necrosis beginning with cytoplasmic degeneration in the immediate vicinity of the nucleus and ending in great, hypertrophied and highly vacuolated, degenerated cells. The earliest morphological evidence of such degeneration is a minute vacuole in an otherwise normal cell (Plate II, Fig. 6). It is conceivable that the material of this vacuolated region is metamorphosed into a substance upon which the salts of the Zenker-fixing agent act to form the curious knobbed filaments. The necrotic cytoplasmic region increases in volume with further degeneration through continued action of the disease, and the fixing material acting on this larger mass produces an increased number of knobbed filaments in a greater cytoplasmic vacuole (Plate II, Figs. 7–10). The ultimate result of this degenerating change is a huge cell skeleton consisting of a well filled out cell membrane, degenerated nucleus, and irregularly distributed material where the cytoplasm had been. The fixing agent, acting on this stage of necrosis, gives few or none of the intracellular knobbed filaments, but, radiating out from the membrane into the surrounding intercellular spaces, may be found masses of such filaments with the characteristic terminal knobs (Plate II, Fig. 2). From such evidence it might be inferred that the necrotic material produced in the degenerating cell had exuded through the cell membrane and was in the course of a general distribution when combined with the salts of the fixing agent. In still other regions the direct connection between the degenerated cell and the deposits is not so close, but throughout the immediate environment may be found the characteristic crystalline aggregates described above as a "running crystalline structure."

If our line of reasoning is correct, it appears that we have in this method of treating cancer tissues, i. e., by the creation of artefacts, a rough means of following morphologically the course of this particular type of degenerative change and the distribution of its products.

From a purely technical point of view, on the other hand, these results point out, in the clearest manner, the need of extreme caution in dealing with Zenker-fixed material. The ordinary precautions advised in every laboratory guide-book are inadequate, and more drastic measures must be employed to clear the tissues and cells of foreign bodies. For this we recommend treatment of paraffin sections for at least thirty minutes with Lugol's solution (potassium iodide plus iodine in water), or treatment of paraffin sections for about two hours with alcohol at a temperature of 50°.

PLATE I.

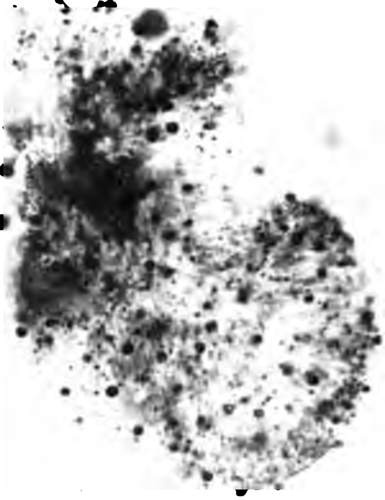


Fig. 1.

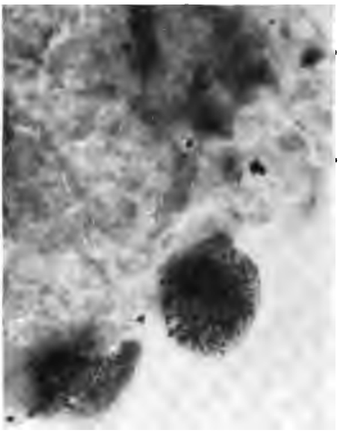


Fig. 2.

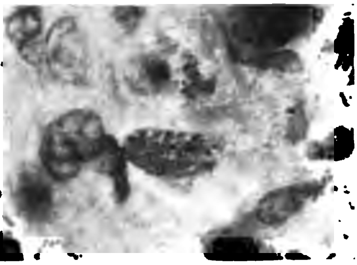


Fig. 3.

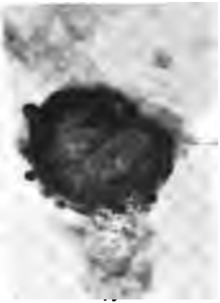


Fig. 4.

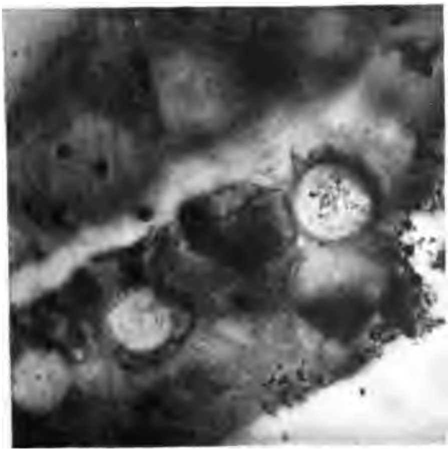


Fig. 5.

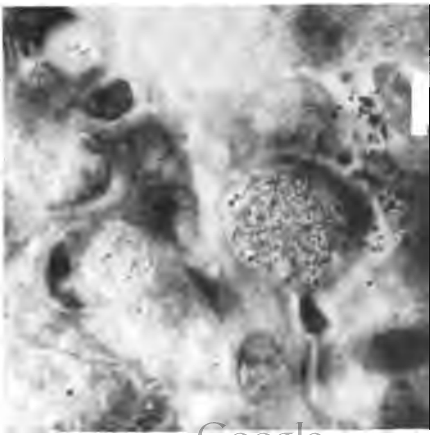
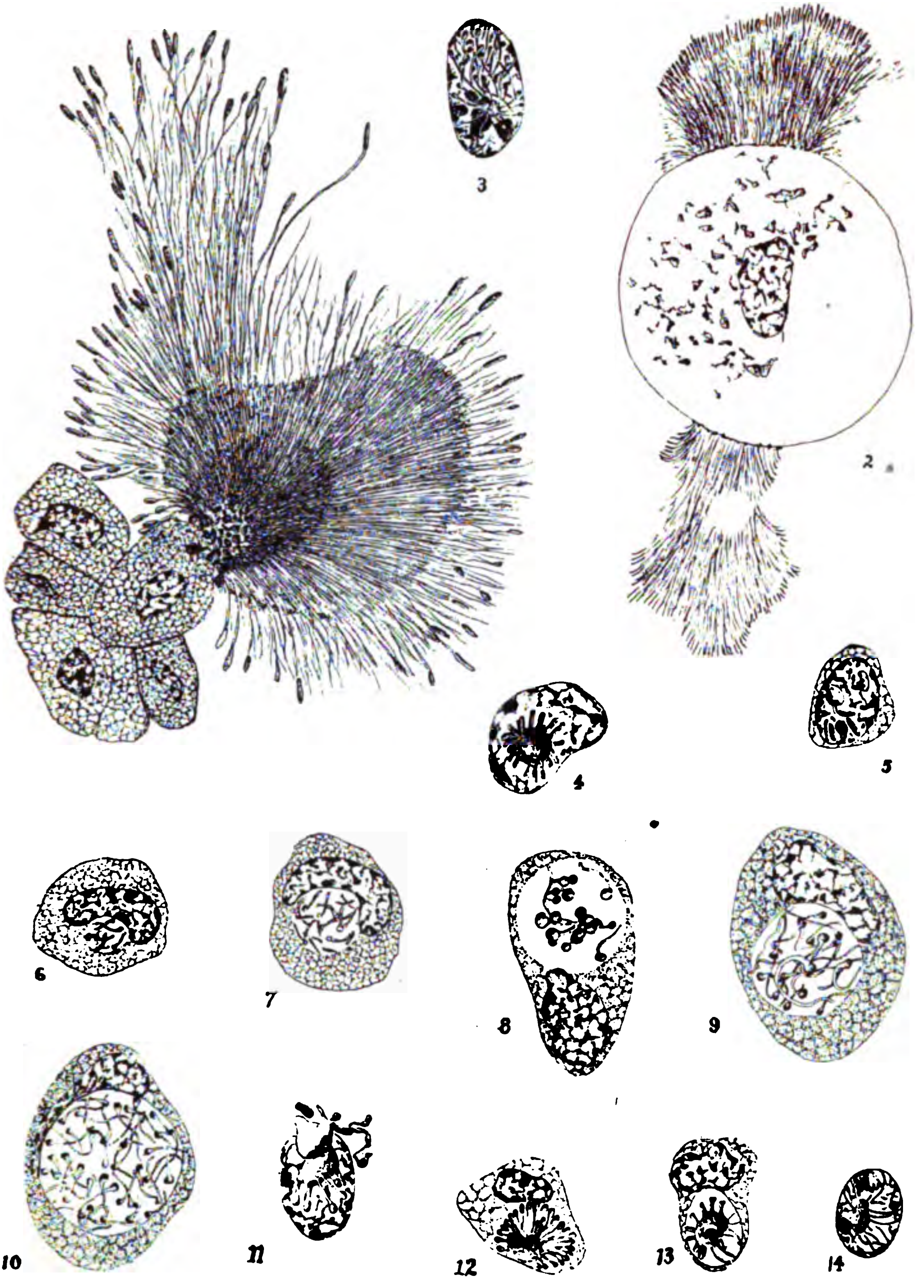


Fig. 6.

PLATE II.



SOME ARTEFACTS IN MOUSE CARCINOMA.

DESCRIPTION OF PLATES.

PLATE I.

(Microphotographs X 1000.)

FIGS. 1 and 4.—Microphotograph of small and large intercellular crystalline deposits, with radiating structure and granules of mercury.

FIG. 2.—Microphotograph of similar inclusions without radiate structure.

FIG. 3.—Microphotograph of nucleus with inclusions (Cf. Plate 18, Fig. 3).

FIG. 5.—Microphotograph of smaller vacuoles and crescentic nuclei.

FIG. 6.—Microphotograph of very large intracellular vacuole, crescentic nucleus and intra-vacuolar deposits.

PLATE II.

(Camera lucida drawings from permanent preparations.)

FIG. 1.—Large intercellular crystalline deposit with radiate structure; the rays extending beyond the periphery are provided with terminal thickenings. × 1800.

FIG. 2.—Degenerate cell with remains of nucleus and flocculent deposit in place of the cytoplasm. From the cell periphery radiating fibers with terminal thickenings reach out into surrounding spaces. × 1000.

FIG. 3.—Nucleus with intranuclear deposits in fiber-form and with terminal knobs. × 1800.

FIG. 4.—Intranuclear deposit. × 1800.

FIG. 5.—Intranuclear deposits of variable size. × 1800.

FIG. 6.—Epithelial cell with beginning of vacuolar degeneration in the depression formed by the crescentic nucleus, and with intravacuolar inclusions. × 1800.

FIGS. 7, 8, 9, and 10.—Epithelial cells with vacuoles of increasing size and with accumulating, characteristic, knobbed inclusions. (Cf. Plate 17, Figs. 5 and 6.) × 1800.

FIGS. 11, 12, 13, and 14, various forms of intracellular and intranuclear deposits. × 1800.

Recent Observations which tend to Support the Parasitic Theory of Cancer.

BY HARVEY R. GAYLORD, M. D.

THE belief that the cancerous process is due to some parasite has come down to us with our earliest knowledge of this affection. In the minds of the earlier observers this was due to the frequent confusion of cancer and certain of the infectious granulomata, especially tuberculosis. The clinical course of many of the sarcomata and the difficulty frequently met with in distinguishing sarcoma in its clinical aspect from such processes as Hodgkin's disease, which is undoubtedly infectious, have sufficed to keep alive, in the minds of many clinicians, the belief in the infectious nature of the malignant processes. It is obvious that a purely clinical point of view may be one-sided, but there is little doubt that many of the theories which have been advanced by pathology have not sufficiently considered the clinical aspects of the disease or else have ignored them entirely. The majority of pathologists are at present distinctly opposed to the belief that any parasite exists which could fulfil the rôle of a parasite for cancer. It is obvious that no ordinary parasite could fulfil this rôle. Therefore, when, in 1886, Scheuerlin, and later Schill, detected bacteria in cancer, it was not long before these organisms were found to be simply harmless saprophytes. This also may be stated to have been the case with the yeast organisms or blastomycetes, which have been more recently described by San Felice and others as occurring in carcinomata.

INCLUSIONS IN CANCER

Since the earliest histological investigations of cancer, there have been observed in the cells certain objects, as to the significance of which much discussion has taken place. It is not profitable to consider here the question whether or not these bodies are parasites. It is interesting to note, however, that as early as 1847 Virchow described these objects, believing them to be metamorphosed nuclei or degenerative changes, of a fatty character, in the protoplasm of the cancer cells. They were again described in 1889 by Thoma, who believed that they were protozoa; in 1890 by Sjöbring and Siegenbeck van Heukelom; in 1891 by Steinhaus;

in 1892 by Soudakewitsch, Borrel, Foa, Kürsteiner, Podwyssozki, and Sawtschenko; in 1893 by Ruffer and Walker and Ruffer and Plimmer; in 1894 by J. Jackson Clarke and Cattle; in 1896 by Pianese; in 1898 by Bosc; in 1901 by E. van Leyden and Gaylord; in 1902 by Feinberg, Greenough, Nösske, and Posner; in 1903 by Apolant and Embden; and in 1904 by G. N. Calkins. Of these observers, Pianese, Greenough, Nösske, and Apolant and Embden believed that the bodies in question are not parasites. The others held them to be protozoa or allied organisms. Calkins holds that, although they have not been proven to be so, they may nevertheless be parasites. The forms in question have come to be known as "Plimmer's bodies," or Van Leyden's "bird's-eye inclusions," or the "*x*-bodies" of Behla. They are spherical structures, which vary in size from four to forty microns. They have a delicate limiting membrane and a central, highly refractive body. The space between the central body and the margin sometimes contains a fine protoplasmic structure, while at other times granules are regularly distributed between the periphery and the central body. They have been observed in the nucleus and in the protoplasm, and in the intranuclear forms they present an appearance not unlike the similar inclusions which have been observed in smallpox and in vaccinia. These bodies have been seen in the fresh state, but they are best demonstrated by complicated hardening and staining methods.

There is no direct proof that these bodies are parasites, although many observers have maintained the belief that they are such. On the other hand, those who have attempted to show that they are not parasites have been forced to employ the same methods of reasoning, and it can be fairly stated that to-day neither those who hold that they are of a parasitic nature nor those who hold that they are not, are in a position to prove their contention. The preponderance of opinion is opposd to the view that these bodies are of a parasitic nature, but this is, to no inconsiderable extent, due to the fact that the majority of pathologists hold, on *a priori* grounds, that cancer is under no circumstances an infectious process. There are some observers, however — notably Borrel — who hold that cancer is an infectious process, that these inclusions are not parasites, but that there is an infective agent in cancer which is either undemonstrable or ultra-microscopic. Perhaps the best arguments in favor of the inclusions being parasites are these: Their similarity in appearance to a known organism — Plasmodiophora brassicæ — and the fact that in certain respects they resemble certain forms of the smallpox organism.

CANCER AND THE ACUTE EXANTHEMATA

Although at first thought there would scarcely appear to be any relation between the cancerous process and the acute exanthemata, yet this analogy between the two groups of diseases has been strongly advocated, principally by Bosc, Gaylord, Borrel, and von Wasielewski; the first two observers basing their advocacy on the ground of the similarity of some the inclusions in the two processes, and Borrel and von Wasielewski on more general grounds.

It will perhaps be of interest to follow more closely the relation which exists between the two processes. Those who discovered a resemblance between the inclusions found in cancer and those observed in smallpox and vaccinia were the first to call attention to the analogy between the two processes. It was Gorini, namely, who first detected points of similarity between certain larger forms of the vaccine body as they appeared in the inoculated corneas of rabbits and the cell inclusions of cancer. This similarity applies only to certain larger forms of the vaccine body which had been previously described by L. Pfeiffer, Guarnieri, and Clarke; but Gorini was able to trace a gradual transition between the larger typical vaccine bodies and these larger inclusions, which resemble the inclusions in cancer. In 1900 the writer observed a similarity between certain of the cancer inclusions and certain forms of the vaccine organism, and from this observation it was inferred by him that if the inclusions in vaccine were parasites, then in all probability the inclusions in cancer were of the same nature. On the same day of the same year Bosc published an article in which he advanced exactly the same idea. At the same time he called attention to the fact that in the lesions of sheep-pox also there were bodies which bore a close resemblance to some of the cancer inclusions. Sheep-pox is characterized by the development of both epithelial and connective-tissue nodules in the subcutaneous tissue.

Bosc found, in the exudate from fresh pustules, characteristic epithelial cells containing highly refractive bodies surrounded by a clear zone of protoplasm; in other words, inclusions closely resembling those described in the epithelial cells of cancer. Similar inclusions were found in the cells forming the connective-tissue nodules. A sheep's cornea inoculated with the virus of sheep-pox presented lesions very similar to those resulting from the inoculations of the rabbit's cornea with vaccine, and Bosc believed that sheep-pox presented an infection lying midway between the malignant epithelial processes and the infectious exanthemata. It is unnecessary to state that the parasitic nature of these inclusions cannot be proved by histological methods alone; and the experiments thus far made with cancer have failed to bring any proof of its specific qualities. On the other hand, the work of Councilman and Calkins and of Bosc and Howard has again brought the significance of the vaccine and variola inclusions into the foreground, and it must be recognized that if these last inclusions — which are apparently incapable of cultivation and which are demonstrated by methods similar to those employed in the case of cancer inclusions, but which present more specific characteristics than do the latter — are ultimately shown to be parasites, then there is a prospect that future investigation may show that the inclusions found in cancer are also of the same nature.

Arguments in favor of the parasitic factor in cancer can, however, be adduced without the aid of these inclusions.

GENERAL ARGUMENTS IN FAVOR OF THE INFECTIOUS NATURE OF CANCER

Transplantation Experiments

Experimental methods in cancer research have opened a new era. This has been made possible by the discovery of the transplantability of tumors in animals of the same species, the first extensive demonstration of which we owe to Hanau, who succeeded in transplanting to the third generation a carcinoma of the rat. Before Hanau, however, as early as 1875, Nowinsky succeeded in transplanting a medullary carcinoma taken from the nose of a dog, successfully in two out of forty-two inoculated dogs. Wehr in

1883 succeeded in transplanting a medullary carcinoma from the vaginal mucosa of a bitch into a number of dogs. Most of these tumors retrograded, but in one animal the tumors grew to considerable size and produced metastases in the adjacent lymph nodes. Following Hanau, Morau in France, Leo Loeb in America, Jensen in Copenhagen, Borrel in Paris, Ehrlich in Frankfurt, Bashford in London, and the New York State Cancer Laboratory in Buffalo have all experimented with the transplantation of primary tumors — mostly in mice, Loeb's first observations being on a sarcoma of the rat. The extent to which this work is now being carried on can be appreciated when it is stated that one tumor alone, that of Jensen, is now being worked upon in at least seven laboratories, and that this tumor has been transplanted to somewhere near the eightieth generation.

The attention which has been attracted to the occurrence of primary tumors in mice has led to the discovery of a large number. Thus Ehrlich has succeeded in collecting tumors in 154 white and 10 gray mice; Bashford collected 9; Loeb has recently detected a spontaneous tumor in a mouse; and the New York State Laboratory is in possession of 8 primary tumors. Borrel has secured in Paris 30 examples of spontaneous tumors in mice, and Haaland speaks of 62 cases known to the authorities of the Pasteur Institute. The latter authority calls attention to the fact that the 62 spontaneous tumors observed in Paris were all in elderly females, and that all of the tumors were adenocarcinomata, involving the abdominal aspect, the axillæ, the groins, or the neighborhood of the anus or the vulva of these mice. They were all derived from the breast. Ehrlich likewise calls attention to the fact that, of 164 spontaneous tumors observed in his laboratory, all occurred in aged females and were all positively derived from the mamma. Eight out of nine of Bashford's mice were elderly females, and the tumors were likewise all derived from the breast; in the one exceptional case — that of a male — the tumor was situated near the root of the tail and presented the same characteristics as the other tumors. Of the eight tumors observed in Buffalo, seven were in females, the sex of the eighth having been unfortunately overlooked. They all presented characteristics similar to those observed in the recognized adenocarcinomata derived from the breast in the mouse.

The fact that all of these tumors were derived from the breast, and the further fact that the mouse appears to be much more frequently affected by carcinoma than are other small animals, can only be explained, as Ehrlich has pointed out, by the facts that all of these mice were obtained from dealers who were engaged in raising white mice for the market and that all the females are employed for breeding purposes. The fact that almost all of the tumors have appeared in elderly females certainly points to the probability that the tremendous demands made upon the mammary tissue of these animals explain the almost exclusive appearance of this form of the tumor. In connection with these facts, the observation of Borrel that healthy mice, when kept for a sufficient period of time in the same cage with infected mice, may develop spontaneous tumors, is of the greatest importance. It has likewise been observed that wherever one spontaneous tumor developed in any particular locality where the mice are being bred, either simultaneously or later, mice with similar tumors have been found.

Communicability of Cancers in Mice

The most striking example of the endemic occurrence of cancer is described by Borrel, who, in the course of two years, observed in one breeding place twenty cases of carcinoma of the breast. All of these mice had, at one time or another, been in the same cage. He observed further, in a second case, in the course of one year, five or six cancer mice, all of which developed in one cage. A similar endemic occurrence of cancer in the rat was observed by Hanau, who first successfully transplanted cancer in this animal. He observed in the course of six years three cases of squamous epithelioma of the vulva. There were in all about one hundred rats, all the offspring of two pairs. Perhaps the most striking evidence of cage infection is found in an observation recently made in the State Cancer Laboratory, combined with a previous observation made by Leo Loeb. Loeb states that in January, 1900, there developed in a group of cages containing rats in the Chicago Polyclinic Laboratory a spontaneous sarcoma of the thyroid. In November of 1901 a second case of sarcoma of the thyroid developed in the same group of cages, and in the autumn of 1903 a third case. The rats had been moved about from cage to cage and were all the offspring of a certain limited number of rats. The tumors presented identical histological characteristics. The first and second rat tumors were used for transplantation, in both cases successfully. On transplantation the tumor presented the characteristics of spindle-celled sarcoma, which in many animals produces characteristic regional and organal metastases. Sections of this tumor have been repeatedly shown at scientific meetings, and there is absolutely no question as to its being a genuine spindle-celled sarcoma. The spring and summer of 1902 were spent by Dr. Loeb at the State Cancer Laboratory in Buffalo. He was provided, for the accommodation of his animals, with two large and a number of small cages. He brought with him a number of rats which had been inoculated from his second sarcoma of the thyroid obtained in Chicago. During the period of his stay in Buffalo he carried out a number of successful transplantations. On leaving the laboratory in September he took with him a number of rats with tumors, but these became infected, and later the tumor was so infected as to be no longer transplantable. After Dr. Loeb's departure all rats were removed from the laboratory. The smaller cages were sterilized in the hot-air sterilizer, but the two larger cages which he had employed, being too large for such sterilization, were simply cleaned and put away. For a period of several months after Dr. Loeb's departure there were no rats of any kind in the laboratory. In the summer of 1903 some rats were purchased in Buffalo for other purposes than tumor transplantation, and a number of them were placed in the two large cages mentioned. In the spring of 1904 there was found in one of these cages a rat with a tumor the size of a horse-chestnut in the subcutaneous tissue of the right abdominal aspect. This tumor was removed by operation, and proved to be a fibro-sarcoma. It was transplanted to other rats, but without success. The occurrence of the development of this sarcoma in the rat was noted and the cage was marked. There were then introduced into the cage a number of adult rats, but, owing to an epidemic of itch among them, it was found necessary to remove the cages containing them to the basement to prevent the possible spread of this infection to the hundreds of mice which occupied the regular animal space in the laboratory. During the summer of 1905 there were found in this cage two adult rats, both males, one with a large fibro-sarcoma in the right abdominal aspect and the other with a large sarcoma of the thyroid. The latter rat died early in October. Sections of the tumor showed it to be identical in appearance with the three primary sarcomas of the thyroid described by Loeb, which developed in the cages in the Chicago Polyclinic Laboratory. In the middle of October an operation was performed upon the other rat. Sections showed that the tumor was a fibro-sarcoma of identical appearance with the one which had appeared in the cage a year before. A number of rats had died during the course of the summer with tuberculosis, so that at the time of the development of the tumors there were but four adult rats in the cage, the two with the tumors and two without. No other tumors have developed in rats in any of the other cages in the laboratory, although the small cages employed by Dr. Loeb and subsequently sterilized have now had rats in them for a period of two years. Aside from the three cases of primary sarcoma of the thyroid developed in Chicago and described by Dr. Loeb, during the period of three years since his departure from the laboratory with his inoculated rats no other author has described sarcoma of the thyroid in the rat, and none has been known to develop in any of the establishments in which these animals are bred. The demand for animals with tumors has become so great that all breeders of white mice and white rats are now on the lookout for tumors, so that the possibility of their having been overlooked is reasonably remote.

Haaland calls attention to a case in which a woman in Paris purchased two white mice for breeding purposes. In the course of two years she sold about two hundred young offspring, reserving the mature mice for breeding purposes. Among these she observed twenty spontaneous tumors. The last three of these mice, with the cage in which they had developed their tumors, were brought to the Pasteur Institute. The mice were removed from the cage and were placed in a new cage, and into the cage in which they had developed their tumors were placed new mice. None of the mice which had previously been in the cage in which the tumor developed, or the new mice which had been placed in it, developed tumors under subsequent observation. The three mice, however, which had already developed sporadic tumors were placed in a new cage, and in this cage were placed with them a number of mice derived from healthy stock, their ancestors so far as known never having had sporadic tumors. Of the healthy mice thus placed in contact with the mice already infected, four developed spontaneous tumors. From this it would appear that to a certain extent these mouse tumors are contagious. If this be admitted, what is the significance of the almost exclusive development of primary carcinoma of the mamma in elderly females among these mice? Ehrlich points out that two explanations are possible. First of all, by reason of the great fertility of the animals the older females are almost constantly carrying and nursing young. It is therefore reasonable to assume that the tremendous demands made upon the breast predispose to an unlimited proliferation of the epithelium of that gland. On the other hand, it is probable that, through the indiscriminate nursing of the young, first by one mother and then by another, an infection of the breast in one mouse might easily be transferred to that of another mouse. It has been shown that in the early stages of carcinoma the breast still possesses the power of lactation, and it is therefore perfectly possible that, through eversion of the nipple, the virus may be transferred from that structure in one animal to the corresponding structure of another. Both in Paris and in Ehrlich's laboratory careful experimentation is being carried on for the purpose of ascertaining whether or not this occurrence can be experimentally proven. In the light of Borrel's observation — viz., that healthy mice which are brought in contact with infected mice can acquire these tumors — it would seem that the exclusive appearance of tumors of the breast among animals used solely for breeding purposes presents very suggestive evidence in favor of an infectious factor.

TRANSFERENCE OF THE INFECTIOUS FACTOR IN CANCER CELLS TO NORMAL EPITHELIUM

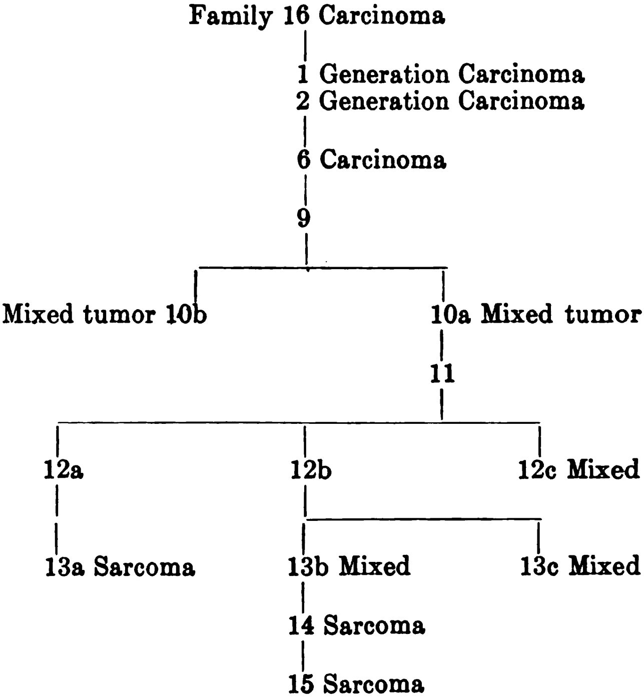
The evidence thus far adduced applies only to primary tumors. If there is a contagious factor which can be transferred from one animal to another, bringing about the transformation of normal epithelial cells into cancer cells, then it is not improbable that, in the very beginning of cancer, this contagious factor may be transferred for a limited period from one cell to the next. In fact, pathologists generally recognize that, in small, beginning carcinomata, such a transformation can be observed. We have from Orth, in his most recent utterance on this subject, the following: "I am, I confess, of the opinion that there are cancers in which the transformation of preformed epithelial cells into cancer cells takes place continuously in the tissue bordering upon the margin of primary tumors; also that there are multicentric cancers, not only in the sense that the cancer change takes place at the same time in different neighboring spots, but also in such a manner that one spot becomes cancerous later than another." If a primary cancer starts from a given centre and the cancerous transformation spreads from cell to cell, it must be that that force or factor which endows normal epithelium with the power of limitless proliferation is transferred, at least for a certain period of time, from the involved cells to the adjoining normal ones. Although this appears to be the case in the period of the inception of a cancer, experimentation has shown that the cancer cell, once endowed with this power of proliferation, retains it most persistently, and a transference of this power to other cells never occurs, unless one or two suggestive observations, which will be referred to later, are evidences of such transference.

Success in transplanting these sporadic tumors in the mice has been variable, but the general experience tends toward more successes as the work progresses. In six of the mice in which cancer was transplanted by Bashford, there were only two in which the disease persisted beyond the second generation. Borrel is in possession of an epithelioma and an adenocarcinoma which are transplantable, and Ehrlich has at present ten different sporadic tumors in process of transplantation, some as advanced as the sixtieth generation. Bashford has had over three thousand transplanted tumors under observation. Ehrlich's, Borrel's, and Jensen's observations must likewise run into high figures, and the New York State Laboratory has already had about six hundred. It will thus be seen that the last two years have been very fruitful in experience in the investigation of cancer, and it may be said that, although the work has just begun, many ideas which we have held regarding this process have been shown to be erroneous, and many characteristics have developed which were entirely unexpected.

CHARACTERISTICS OF TRANSPLANTABLE MOUSE TUMORS

It must be pointed out that the mere transplantability of cancer throws no light upon the mechanism by which spontaneous tumors develop. These transplantations are modifications of the process of metastasis. The success with which they have been accompanied has shown great variability, but on the whole the experiences of all laboratories have been that tumors which have been often transplanted acquire an increased virulence, so that, although the success attending the first attempts at transplantation has in many cases been as low as one or two per cent., in later cases the virulence has risen to such an extent that the average has been as high as from eighty to one hundred per cent. A most interesting example of the tremendous virulence of these transplanted tumors is found in one which is under observation by Ehrlich. This, known as No. 7 in his series, presents a virulence which is most astonishing. The transplantations with this tumor material have for a considerable period of time given from eighty to one hundred per cent. of successes. The transplanted tumor grows with such rapidity that in eight days after inoculation it has been found to weigh 2 gm.; at the end of two weeks, over 3 gm.; and at the end of three weeks, usually 5 gm. Tumors as large as the mouse itself not infrequently develop within two months from the time of inoculation. All laboratories which have been working on transplantation have had similar experiences. Some tumors are found to grow very slowly, as did that of Morau, which required months for its full development, whereas others present the characteristics of the tumor described above. In all tumors, however, repeated transplantation, instead of weakening the energy of the tumor, seems to increase its virulence, and it is now recognized that the most distinguishing feature of cancer is the unlimited power of proliferation which the cancer cells possess, this power having already carried some tumors beyond the sixtieth generation of transplantation through healthy mice.

All this experimentation has failed to show us how the cancer cells acquire this phenomenal power of proliferation. That the characteristic factor of cancer is found only in the epithelium is shown by the fact that the stroma in the transplanted tumors is furnished by the host. That this factor, in the course of transplantation of mouse tumors, is occasionally transferred to the connective-tissue elements of the stroma, endowing them with sarcomatous characteristics by which the tumor is transformed into a mixed tumor, is shown by the fascinating publications of Ehrlich and Apolant (*Berl. klin. Wochenschr.,* 1905, No. 28, and 1906, No. 2). These observers have now encountered this phenomenon in three tumors. In the first case observed, the tumor presented the usual characteristics of the adeno-carcinoma of the mouse and had been transplanted without any change to the sixth generation. The tumor consisted of nests of varying sizes of alveolar arrangement, with a not very well developed connective-tissue stroma. Between the sixth and ninth generations the tumor underwent a change in which the carcinoma suddenly presented the characteristics of a mixed tumor, the thin connective-tissue stroma presenting every evidence of active proliferation; wide avenues of closely packed, deeply staining spindle cells, with abundant karyokinetic figures, appearing between the nests of epithelium. These characteristics persisted from the ninth to the thirteenth generation, the epithelial characteristics gradually diminishing and the nests becoming smaller and more widely separated; and in the fourteenth generation the epithelium had entirely disappeared from the tumor, leaving a spindle-celled sarcoma, which is still being transplanted and has reached the fortieth generation. The accompanying table from Ehrlich will serve more graphically to emphasize this remarkable observation:



The generations marked a, b, c descended from various mice of the preceding series.

Recently Apolant and Ehrlich have reported two further similar observations. In one of these the sarcomatous transformation developed in an adeno-carcinoma which was provided by mixing together various adeno-carcinomata which were respectively in the twenty-first, the thirty-third, the twenty-third, and the nineteenth generations of transplantations. One of the strains derived by this mixture, between the twelfth and fourteenth generations, showed a marked increase in the proliferation of the connective-tissue stroma, which awakened at once the suspicion that the development of sarcoma was taking place. In the sixteenth generation this was so far developed that the tumor presented the characteristics of a mixed tumor. In contrast to the first case reported, the differentiation between the nests of epithelial cells and the proliferating stroma was not nearly so marked as in the preceding case. The sarcoma cells likewise were more polymorphous in appearance, typical spindle cells forming only a part of the tumor. They lay in irregular masses that filled the spaces not occupied by the net-like structure of the epithelial portion of the tumor. The proliferative characteristics of the sarcomatous portion of the tumor were not nearly so marked as in the first case. The tumor at present is in its tenth generation of transplantation, and the proliferation of the connective-tissue and epithelial elements appears to be about on the same footing as it was before, the tumor having during the last six months shown but slight changes in the relative proportion of epithelium and connective-tissue. The rapidity of growth of this tumor shows no diminution, the authors having observed tumors of enormous size, in many cases equal to that of the mouse itself.

The third observation is the most striking of all. It occurred in the course of transplantation of Ehrlich's tumor No. 7, which is the most virulent of all mouse tumors yet under observation. This tumor had shown, from the fortieth to the sixty-eighth generation, a marked increase in the connective-tissue without the stroma presenting the characteristics of a sarcoma, when suddenly in the sixty-eighth generation it took on a marked sarcomatous appearance, associated with such colossal proliferation that in the next generation many of the tumors were sarcomas without any evidence of epithelioma. Here and there some of the transplanted tumors contained a few nests of epithelium. These remnants of carcinomatous epithelium have been detected as late as the seventy-first generation. The sarcoma cells in this tumor were likewise more polymorphous in character, those of a spindle shape being in a minority. This tumor has now been transplanted three generations further without any loss of the colossal proliferative qualities with which it has been endowed from the first. The explanation of this phenomenon given by Ehrlich is that some form of stimulus present in the carcinoma cells is in certain phases of its development transferred from the epithelium to the connective-tissue stroma of the tumor and transforms the connective-tissue cells of this structure into typical sarcoma cells capable of probably indefinite transplantation.

It is impossible to draw conclusions from a single observation, but the phenomenon described above may possibly be explained by the assumption that the chief characteristic of a cancer — viz., its power to proliferate to an unlimited extent — has, in this particular instance, been transferred from the epithelium to the connective-tissue. To assume, on the other hand, a transformation of epithelial cells into connective-tissue cells, would be contrary to all our histological knowledge. It can, of course, be said that the phenomenon under consideration represents merely the sporadic development of a sarcoma on the basis of a carcinoma. That the *x*-factor in cancer may possibly be transferred to other cells is shown by the frequent observations referred to by Haaland, and observed in Buffalo, of the development of primary adenomata in the lungs of mice which have been the subject of transplantation of these mammary tumors. Haaland refers to the fact that this primary development of adenomata in the lungs of mice is a not uncommon occurrence, and our own observations corroborate this statement.

NATURAL IMMUNITY TO IMPLANTATION IN MICE

It has been found in all laboratories that a certain proportion of mice cannot be inoculated with the tumor. Thus far, a natural immunity against these inoculation experiments appears to bear no definite relation to heredity, but in all laboratories mice have been found which appear to be permanently immune, and these mice have frequently been the offspring of parents both of which were afterward successfully inoculated and died of the tumors.

SPONTANEOUS RETROGRESSION IN CANCER OF THE MOUSE

Although the disease, once established by implantation, is in a very large per cent. of the cases fatal, in all laboratories occasionally spontaneous cures have occurred. These have been observed in Erhlich's laboratory and also by Bashford; and apparently, up to the present time, the greatest number have occurred in the Jensen mice under observation in the State Laboratory in Buffalo. Immediately following the transplantation of these tumors — which is done by taking uncontaminated tumor, mixing it in a mortar with three or four parts of normal salt solution, and injecting it beneath the skin of the back through a coarse needle with a syringe or introducing particles through a small trocar — there is frequently a slight reaction, which subsides on the second or third day. It is obvious that in many of these experiments transient infection occurs, as shown by the formation of an abscess. This usually interrupts the experiments but occasionally the swelling subsides and ultimately a tumor develops.

In the period from February to June, 1905, not less than twenty per cent. of the tumors resulting from successful inoculation underwent spontaneous retrogression. This is a higher percentage of spontaneous recoveries than has yet been reported from any other laboratory. The distribution of the period in which the processes of retrogression were apparent shows that more spontaneous retrogressions occurred early in the process than late. There are, however, a certain number of retrogressions which occurred in what would normally be the last stages of the disease. One of the most striking examples occurred in a rapidly growing tumor, which reached a weight of over 3 gm. in forty-three days after the inoculation, then began to retrograde and ultimately disappeared.

That a spontaneous cure of a genuine carcinoma in the mouse should occur and should be well authenticated would at first seem surprising, but a careful review of the literature has shown that undoubted cases of spontaneous cure have also been observed in human beings. It is natural that a greater percentage of these cures should occur under experimental conditions than under the conditions in which we encounter cancer at the bedside. Mice used for experimentation are taken at random, and it is obvious that some of them have a greater resisting power than others, as shown by the fact that a certain percentage of them possess a natural immunity which protects them from being successfully inoculated. The cases which we meet clinically are those of individuals who apparently have no sufficient immunity, and we see therefore only the unfavorable cases. It is not improbable, however, that even in human beings patients become infected with cancer, but make early spontaneous recoveries, perhaps without attracting even their own attention.

EVIDENCE OF AN ACQUIRED IMMUNITY AGAINST CANCER IN MICE

Researches in the State Laboratory as to the nature of the phenomena associated with spontaneous cure point very strongly toward the presence, in mice which have recovered spontaneously, of a form of acquired immunity. This is shown by the failure successfully to reinoculate any mouse which has spontaneously recovered. The immune factor is apparently present in the blood, and in some mice has been sufficiently active, when injected into other mice with growing tumors, to influence the growth of the tumor. In this way small tumors have been made to retrograde and large tumors have been inhibited in their growth. Further proof of the presence of an immune factor in the blood of mice is found in the recent observations of Clowes, which show that when cancer material is treated with a sufficient proportion of the blood of spontaneously recovered mice the number of successful inoculations is markedly reduced.

HISTOLOGICAL CHARACTERISTICS OF RETROGRADING MOUSE TUMORS

Examinations of the histological appearance of tumors undergoing spontaneous retrogression, and of those retrograding under the influence of injections with immune serum, show identically the same picture. If the action of this serum were cytolytic in its nature, we should expect to find evidences of destruction or direct

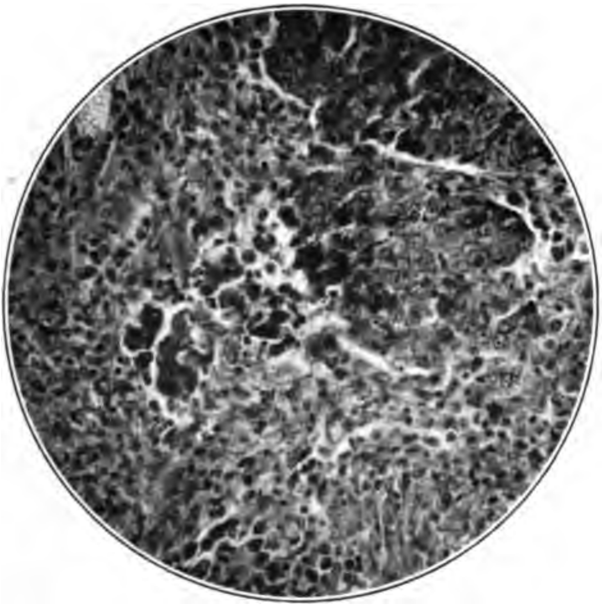


FIG. 1.—MICROPHOTOGRAPH. x-260. EPITHELIUM AT MARGIN OF TUMOR UNDERGOING RETROGRESSION FROM x-RAY TREATMENT.

injury to the cells, but this is not the case. About the margins of retrograding tumors one finds that the cells have undergone simple atrophy, and that where groups of cells remain they frequently coalesce into pseudo-giant cells. These are surrounded by connective tissue, and ultimately, through the process of atrophy, disappear. In tumors undergoing retrogression hemorrhage is a frequent occurrence. An examination of the cancer cells immediately adjacent to the hemorrhages in the tumor shows that this process of simple atrophy is most marked where the cells have come in contact with the extravasated blood. Practically, one can see here the direct action of the blood upon the cells. There is no necrosis of the protoplasm, and karyokinetic figures can be found in the epithelial cells until the very last. The picture presented shows that the epithelial cells are subjected to a process which is identical with that which overtakes transplanted or misplaced normal epithelium. Leo Loeb and others have shown that if fœtal epithelium is aseptically transplanted into the subcutaneous tissues in adult animals, it is able to maintain itself for a period of time during which its dynamic force suffices for proliferation to the sixth or seventh generation, after which the force is expended, the cells undergoing atrophy and becoming surrounded by connective tissue, which grows between them. The picture presented here is exactly like that found in these retrograding tumors.

From this observation it must seem obvious that in spontaneously retrograding tumors the immune factor, instead of working directly upon the cells, reduces them to the status of normal epithelium, and they are then removed by a process of atrophy and repair which is identical with that which overtakes misplaced normal epithelial elements. Becher, Peterson, and Schwartz have shown that similar reparative processes are frequently at work in many human carcinomata. That the connective-tissue activity is secondary is shown by inference with the immune mechanism, which inference can be brought about by bleeding. In the New York State Laboratory it has been found that in the case of tumors which were undergoing retrogression as the result of injections of immune sera, severely bleeding the mouse would interrupt the process and the tumor would thereupon begin to grow as rapidly as ever. This observation, in connection with the facts which tend to show that the immune factor is in the blood, strongly indicates that the proliferation of the connective tissue is but a secondary process, which only becomes active when the cancer cells are reduced to the status of normal epithelium.

IDENTITY OF HISTOLOGICAL CHARACTERISTICS OF SPONTANEOUSLY RETROGRADING TUMORS AND TUMORS RETROGRADING THROUGH TREATMENT WITH IMMUNE SERA, THE x-RAY, OR RADIUM.

The changes which are brought about in carcinoma, either in man or in animals, by exposing them to the activities of the *x*-ray or of radium, have been shown by Exner, Perthes, and others in man, and by Apolant and Embden and Bashford in mice, to present exactly the same histological picture as that which is presented by tumors undergoing spontaneous retrogression. This fact has been under observation for over a year in Buffalo. A section of a tumor retrograding under the activity of the *x*-ray or of radium is in no way distinguishable from one taken from a tumor undergoing spontaneous retrogression or retrogression induced by serum treatment. Examination of the blood of mice which have recovered from tumors through the activity of the *x*-ray shows that this fluid does not contain any acquired immune factor. If, however, in the course of treatment a mouse is heavily bled, the tumor will frequently begin to grow — a phenomenon which leads to the conclusion that the *x*-ray does not act directly upon the tumor, but through such immune factors as the mouse still possesses. For this reason it would appear that the *x*-ray and radium reduce the virulence of the tumor or so injure the *x*-factor that the natural immunity brings about the retrogression of the tumor. In this way it is possible to explain those tumors which are not affected by the *x*-ray, and also the fact — which has been frequently observed — that tumors which are being favorably affected suddenly begin to grow in spite of continued treatment.

SIGNIFICANCE OF PERTHES' EXPERIMENTS WITH THE x-RAY ON WARTS

That the *x*-ray, either directly or, as would appear, indirectly, robs the epithelial cell of the factor which causes its unlimited

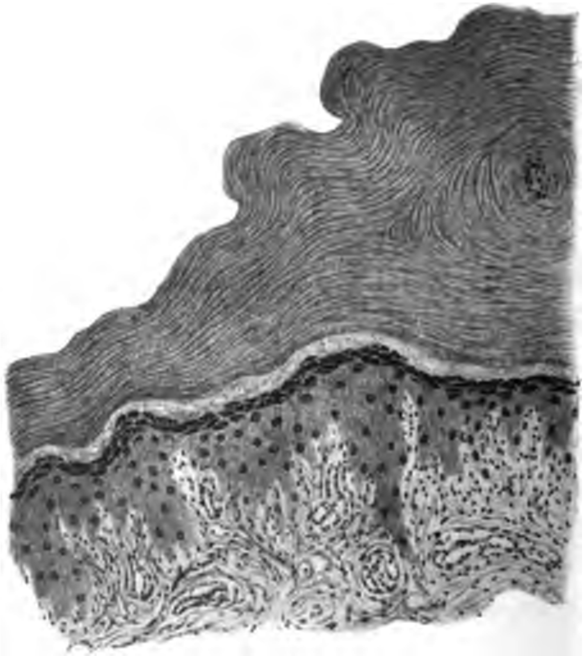


FIG. 2.—SECTION OF WART THIRTEEN DAYS AFTER FIRST TREATMENT, NINE DAYS AFTER LAST TREATMENT WITH X-RAY, SHOWING COMPLETE HORNIFICATION OF EPITHELIUM OF WART AND NEW SKIN FORMED FROM DEEPER LAYERS. (*Perthes.*)

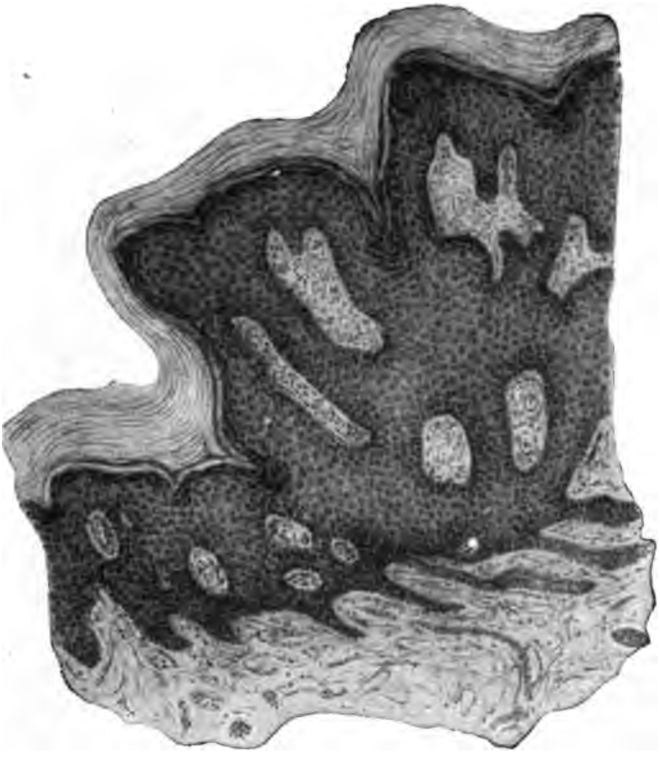


Fig. 3.—Section of Untreated Wart for Comparison. (*Perthes.*)

proliferation, and leaves the normal epithelial cells unaffected, is shown by the interesting experiments on warts by Perthes. Perthes has clearly demonstrated that the dose of x-ray required in the treatment of cancer, or for the removal of warts, does not injure directly either the normal epithelium or the epithelial cells of the tumor. If the tissues surrounding the tumor are overdosed a so-called x-ray burn may be induced, but this is an injury entirely independent of the ideal therapeutic activity of the agent. Perthes has shown by sections that a wart which has been properly dosed, frequently with but one treatment, will thereupon undergo a process of retrogression, in which all of the cells forming the wart become hornified, *with the exception of those of the deeper or germinal layer; and these promptly proliferate and produce, not a new wart as before, but normal new skin to repair the defect.* If the dose is not sufficient the superficial cells will undergo hornification, the wart will be reduced in size, but the cells of the deeper layer will again proliferate and produce a new wart. *This proves conclusively that the x-ray does not act through any form of injury to the cells themselves. It removes from them the tendency to proliferation which produces the wart, and leaves behind, in the necessary cells of the germinal layer, normal uninjured cells which are capable of producing new and normal skin.* As it is the fate of superficial epithelial cells of the skin, when their period of utility is passed, to undergo hornification, the process of hornification in the cells of the wart is probably secondary. Once their power of abnormal proliferation has been removed, they succumb to that fate for which they were normally intended, which is hornification; and this involves all of the cells of the wart except those which are destined to resume their normal functions.

CHARACTERISTICS OF THE UNKNOWN STIMULUS IN CANCER

The observations thus far accumulated on the spontaneous retrogression of tumors, the retrogression of tumors through an immune agent, and the direct or indirect activity of the x-ray and radium, tend to show that in these agents we have a means of removing from the cancer cell the x-factor. If, as conceded by Orth, there takes place at the margin of tumors a gradual transformation of normal epithelial cells into cancer cells, and if, by the action of immune sera and the *x*-ray, we can again reduce these cancer cells to the status of normal cells, it seems almost conclusively shown that there can be added to a normal epithelial cell a factor which is capable of endowing it with the power of continuous proliferation, and which can again be removed from it, leaving a normal epithelial cell. This normal epithelial cell, it is true, may be superfluous, in which case it will undergo processes of atrophy and removal the same as may take place in any other misplaced normal epithelial cell. But if, as in the case of warts, the cell still has a function to perform, it can resume its natural proliferative activity — an activity which does not overstep the bounds set by the physiological laws of normal life.

It has been suggested that the unknown factor in cancer is of a chemical nature. No less an authority than Marchand has suggested that it might be some toxin. If the facts in the case are considered, it is obvious, as Clowes has shown, that this is impossible. An agent which is capable of keeping up continuous proliferation in cancer cells — which, theoretically speaking, starts with one cell and passes into its offspring through thousands of generations, during which time the number of cancer cells increases indefinitely — must, quantitatively speaking, increase in bulk. That this must be so is evidenced by the fact that this factor can be removed and must be removed from each and every cancer cell before the cell undergoes retrogression. This is shown to be the case in experimental tumors which are undergoing retrogression after treatment by the *x*-ray or through the activity of sera. The changes brought about by either of these agents is found to be most marked at the periphery of the tumor, and it has been found that epithelial cells taken from the centres of tumors which are retrograding at the margins can be transplanted and will produce tumors, whereas the cells at the margins present changes which show that this would be impossible. Therefore, the agent must be removed from each and every cancer cell; and as this agent, although present in the beginning in but one or two cells, later comes to occupy a bulk of cells which can scarcely be estimated, and as, furthermore, it must likewise have increased rather than have diminished in activity, it must certainly also have increased in amount. How enormous the proliferative

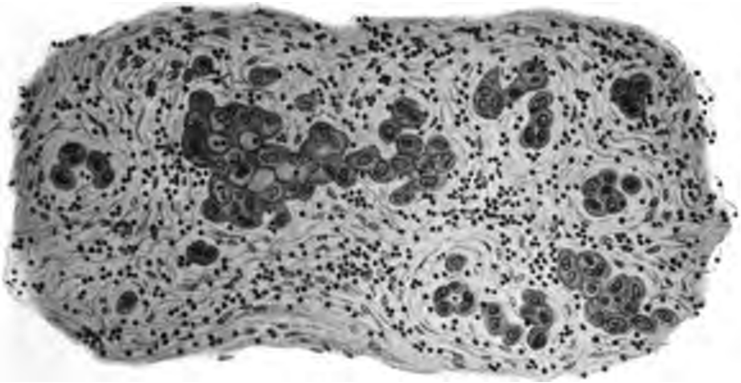


Fig. 4.— Human Tumor Undergoing Retrogression from Treatment with x-Ray. (*After Perthes.*)

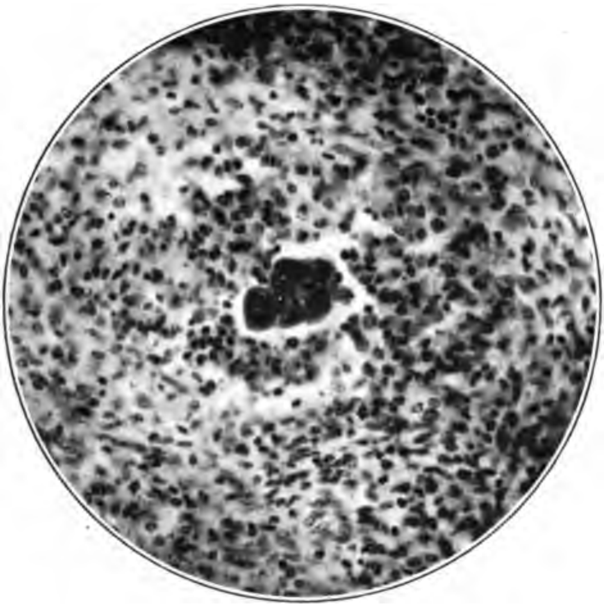


FIG. 5.—MICROPHOTOGRAPH. x-260. LAST REMNANT OF EPITHELIUM FROM A TUMOR UNDERGOING SPONTANEOUS RETROGRESSION.

powers of even a small mouse tumor may be is indicated by the astonishing figures which Ehrlich has published in connection with his rapidly growing tumor No. 7. He has estimated that this tumor is now growing at a rate which would permit of its being carried through sixty generations in one year. As the tumor is now giving nearly 100 per cent. of increase, he estimates that if from 12 to 15 mice were used for each transplantation, within one week 10 tumors of the size of that used for transplantation would be produced. From each of these in eight days 10 more could be produced, so that in the third generation 1,000 tumors, in the fourth 10,000 tumors, and so on, would result. If this were carried to the sixtieth generation, it would represent 10^{60} c.cm. of tumor if each tumor weighed but 1 gm. In the course of one year this would lead to a bulk of tumor which is scarcely comprehensible. It represents, according to Ehrlich, a cube the edge of which would measure 1,000,000,000,000 kilometres — a distance which it would require light 105 years to traverse. In spherical form it would represent a mass with a diameter 890 times greater than that of the sun, and a volume exceeding that of the sun $7\text{x}10^{26}$. If the agent which could keep pace with this tremendous increase in bulk were a toxin, it could only do so by reproducing itself; and the only possible mechanism by which this could be brought about would be by the agent acting upon the protoplasm of the new cell in such a manner as to cause it to produce its like. No chemical agent, however, with which we are acquainted, toxin or otherwise, and which is capable of bringing about a reaction in living protoplasm, causes this protoplasm to produce the same agent. On the contrary, the protoplasm produces, in all cases thus far known, an agent which is antagonistic to the first — in other words, some form of anti-body. For this reason it is impossible to conceive of any chemical agent endowed with the power to fulfil the conditions of the *x*-factor. We are therefore compelled to assume that the *x*-factor must be some agent which can reproduce itself, and thus far the only agents with which we are acquainted which can accomplish this are living agents. Hence the most rational explanation of the unknown factor in cancer is that it is some living agent. If we so wish we can speak of this agent as a virus, as does Borrel, inasmuch as we do not know its specific nature. Borrel believes that there is an infectious factor in cancer as yet undemonstrated, and that it is in all probability an invisible or ultra-microscopic organism. The same contention has been made in the case of syphilis, because the agent was unknown (unless the recent observations of Schaudinn, and of many others confirming it, should ultimately show that spirochæte pallida represents a phase of the organism of syphilis); and it is likewise held to be true in smallpox, in vaccinia, and in other diseases.

SIGNIFICANCE OF FILTRATION EXPERIMENTS

The belief that the contagious factor is invisible has usually been based upon filtration experiments. The virus of sheep-pox has been shown by Borrel to pass through the Berkefeld and the coarser grades of the Chamberland filter. This has likewise been shown to be the case with vaccine virus. The question arises as to whether filtration experiments are necessarily an evidence of an ultra-microscopic organism. Borrel furnishes light on this point. In his filtration experiments with sheep-pox he discovered that when he diluted the virus with tap water, after four days there developed in the filtered and otherwise sterile virus a small protozoön, to which he gave the name Micromonas mesnili. The organism when in its largest form in the virus — it is of course impossible to affirm that the organism under other conditions does not possess a still larger phase — was three or four microns long and as many wide. That the organism in question had nothing to do with the virus was shown when distilled or sterilized water was used to dilute the virus. Borrel found that his organism followed the same law as the active principle of the virus; that is, it passed through the filters through which the virus passed, and was held back by filters which were proof against the passage of the virus. That the organism passed through in some practically invisible spore form, and then developed on the suitable medium of the virus, was shown by its appearance in virus only after four days and the impossibility of detecting it in filtered water in which the larger forms did not develop. Borrel was forced to conclude: "Le passage à travers un filtre n'implique pas forcément l'idée d'un microbe invisible."

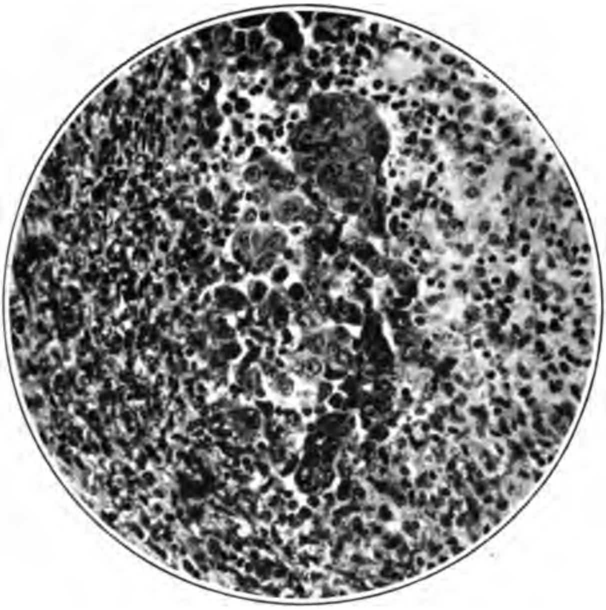


FIG. 6.— MICROPHOTOGRAPH. x-260. ALVEOLUS AT MARGIN OF TUMOR UNDERGOING RETROGRESSION FROM SERUM TREATMENT.

It must be noted that Borrel's Micromonas shows as its largest form an organism considerably smaller than the larger inclusions of vaccine, variola, and cancer. However, to assume that because the spore of an organism is sufficiently small to pass through a certain filter, its largest form would be within a certain limit of size, is not justified by biological knowledge. Calkins has described a protozoön, Lymphosporidium truttæ, the spores of which have a diameter of one and one-half microns and divide into six sporozoites,. each less than one-half a micron in diameter. Borrel likewise points out that there is an essential difference between the smaller forms of motile animate parasites and bacteria of relatively the same dimensions. The first are more plastic and accommodate themselves to the pores of the filter, passing through where bacteria are held back. In all probabiltiy the sporozoites of Lymphosporidium truttæ, less than one-half a micron in diameter, would pass through a bacteria-proof filter; and yet the largest form of this organism is a multinuclear amœba twenty-five microns in diameter.

It will be noted that Micromonas mesnili is three to four microns long and as many wide. The spirochæte pallida in its smallest form is one-quarter of a micron in diameter and four to fourteen long. Becchi has shown that even large protozoan amœbæ, twenty-five microns in diameter, may pass readily through Berkefeld bougies, and for this reason it is desirable to eliminate filtration experiments in attempting to determine the relative size of organisms. There is a not remote similarity between the subcutaneous lesions of syphilis and some of the infectious granulomata and even sarcomata.

Infectious Venereal Granuloma of the Dog

In this connection Bashford has recently described the histological characteristics of an inoculable venereal granuloma found in dogs, which possesses certain characteristics of a malignant tumor, and presents still others which leave no doubt that it is an infectious process, although the virus or organism is as yet undetermined. This tumor seems to be almost a connecting link between the infectious granulomata and malignant tumors. It is common in the dog, is transmitted by coitus, and develops in the subcutaneous tissue about the genitals. The tumor cells are polygonal, with scanty granular protoplasm and large, spherical nuclei. In the resting stage they possess one large nucleolus and a delicate chromatin reticulum. Mitotic division is common in the nuclei, and, although the type is commonly bipolar, *multipolar figures* are not unusual. The tumor is divided up into lobules by delicate connective-tissue septa containing fully developed capillaries. Hemorrhages are frequently found. The general appearance closely resembles that of a round-celled sarcoma with a parenchyma arranged in alveoli. In primary tumors (see Fig. 7) Bashford shows that a transformation of the connective-tissue cells into tumor cells can be demonstrated. This is only apparent where the rapid growth of the tumor has not resulted in pressure upon the surrounding structures. One striking feature of the tumor is that, although this transformation is going on at the periphery, the greater portion of the bulk of the tumor is brought about by proliferation of the tumor cells, in this way closely resembling the method of growth of a true malignant tumor. In the later stages the tumor grows almost entirely from its own resources. The primary lesion in this tumor is therefore in no way different from that of a primary sporadic carcinoma, in which there is likewise a transformation, at the margin, of normal cells into cancer cells. When, however, this tumor is transplanted, its true characteristics appear. According to Bashford, when the tumor cells are implanted in the subcutaneous tissues of a new host, all of the implanted cells, instead of continuing to proliferate, disintegrate, and a new tumor is formed by the action of the specific factor upon the connective-tissue cells of the host. Evidence of this process is found by Bashford at the margin of newly developing nodules after implantation. Inasmuch as Bashford believes that the implanted cells all disintegrate, it is obvious that the virus is the only factor which persists.

All attempts to determine the precise nature of this virus have thus far failed. Filtration experiments do not appear to have been carried out thus far. These tumors frequently grow to great size and sometimes undergo spontaneous retrogression. In transplantation experiments new tumors can be recognized

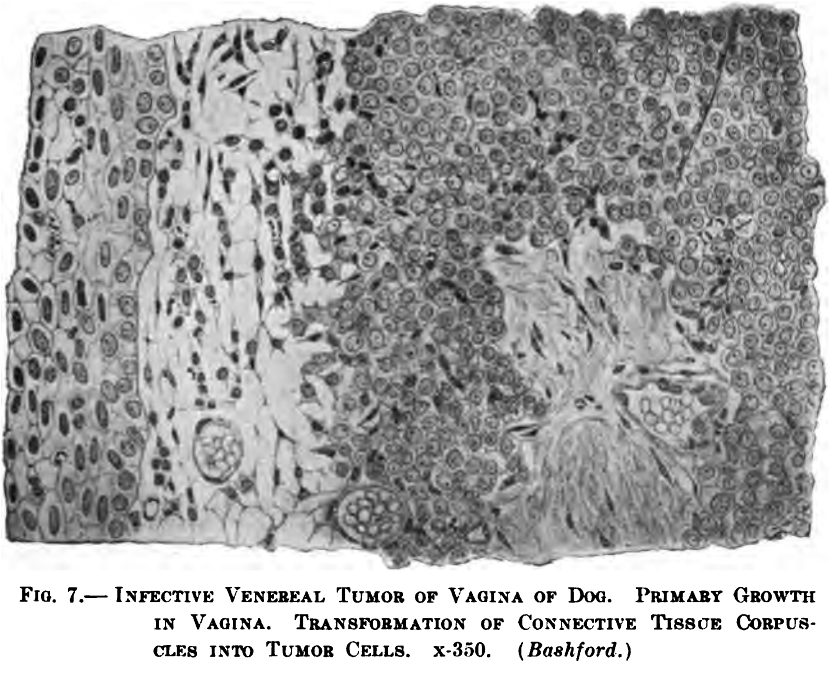


Fig. 7.— Infective Venereal Tumor of Vagina of Dog. Primary Growth in Vagina. Transformation of Connective Tissue Corpuscles into Tumor Cells. x-350. (*Bashford.*)

in from eight to ten days, and subsequently they attain a diameter of several inches. Metastases may likewise develop in the mesentery after intrascrotal inoculation, and the lymph nodes adjacent to large growths are frequently enlarged. The disease cannot be transmitted to the cat, rabbit, guinea-pig, or mouse. Bashford concedes that, in its histological features, local mode of origin, partial growth from its own resources (in the later stages causing pressure on surrounding tissues and organs), and in the limitation of its transmissibility to one species, it closely resembles a malignant growth. He believes that its invariably infective history, the transformation of the surrounding connective-tissue corpuscles into tumor cells even in fully developed tumors, its artificial transmission, following the laws of such granulomata as tubercle or glanders, and the fact that it occurs naturally in animals before sexual maturity, all serve to distinguish it from true malignant tumors.

These objections, in the light of the facts already adduced, are not very convincing. On the other hand, we have shown that there is strong evidence of an infection in the primary sporadic tumors of the mouse, and we may add that many authorities concede that in primary tumors there is a transformation of normal epithelial cells into malignant epithelial cells at the margin of the tumor. Furthermore, the fact that the disease occurs naturally in young animals, which is likewise true of sarcomata, should speak rather in favor of an analogy to malignant growths, which appear oftener in old age than otherwise. The one respect in which they appear to differ essentially from malignant tumors is that the cells do not appear to possess the power of limitless proliferation. It would seem that in transplantation experiments it would be a matter of great difficulty to determine whether or not all of the implanted cells disintegrate, and the essential point in which these tumors appear to differ from malignant tumors seems to be in the transformation of the normal cells of the host, in transplanted tumors, into tumor cells. The transplanted tumor in this case appears to repeat processes which are found in the development of sporadic tumors only, and as such it would appear that this tumor should in the future be the source of much fruitful investigation. The points which it has in common with true malignant tumors, the fact of its invariable infectivity, and the undoubted presence of an infective factor, should throw much light upon the much more elusive factors in malignant tumors.

Sticker, who has carried out extensive transplantation experiments with a tumor similar to the one described by Bashford, and who has carefully compared those transplanted by Smith and Washburn, Wehr (1888), and Geissler (1895), arrives at the conclusion that Bashford's interpretation of these tumors is not correct, and that they are genuine round-celled sarcomas. In this he is supported by Albrecht, Bollinger, Duerck, von Hansemann, Kitt, Luepke, Orth, Ribbert, Schmaus, Schmorl, Schuetz, Arnold, and Weigert, all of whom examined specimens of all five tumors (Smith and Washburn, Sticker, Wehr, Geissler, and Bashford), and diagnosed them to be typical round-celled sarcoma.

SUMMARY

The following, then, are the arguments which have been adduced, from the modern research into cancer, in favor of the infectiousness of the process:

1. An analogy exists between certain of the changes in the epithelium in cancer and those occurring in the epithelium in certain of the acute exanthemata, notably variola and sheep-pox, known infectious diseases.

2. The almost exclusive appearance of cancer of the breast in elderly female mice which have been used extensively for breeding is best explained by the transference of some infective agent, through the medium of indiscriminate nursing, by offspring (Ehrlich).

3. Tumors in mice are almost never found alone. In breeding establishments, where one case appears it is always accompanied by others. Healthy mice, brought in contact with mice with primary tumors, acquire the same (Borrel).

4. The reappearance of sarcoma of the rat in a cage which had contained rats inoculated with sarcoma points to the possibility of cage infection in this form of cancer.

5. A gradual transformation of normal epithelial cells into cancer cells occurs at the margins of primary cancers (Orth).

6. The continued transplantation of mouse tumors increases rather than reduces their virulence. Certain mouse tumors under transplantation have acquired a virulence only comparable to that of an acute infectious process.

7. The transformation of an adenocarcinoma into a sarcoma (Ehrlich) is most easily explained by assuming the transference of an infective factor from the epithelium into the connective tissue of the stroma.

8. A certain number of mice are shown to possess a natural immunity which prevents inoculation with cancer. Spontaneous retrogression of cancer in mice is accompanied by histological appearances which show that the epithelium is not primarily injured, but that the stimulating factor is removed. Spontaneous retrogression is accompanied by a type of acquired immunity which prevents the successful reinoculation of the animal, and under favorable conditions this factor appears to be present in the blood and behaves not unlike the known antitoxins to infectious processes.

9. The blood of spontaneously recovered mice, when added to cancer material before transplantation, removes from it the power of continued proliferation. There is no evidence of cytolytic action (Clowes).

10. Tumors retrograding under the influence of the *x*-ray and radium present exactly the histological picture of tumors spontaneously retrograding. The stimulating factor seems to be removed from the epithelium through the aid of the immune mechanism.

11. The epithelial cells of the deeper layers of warts, after successful treatment with the *x*-ray, no longer proliferate to form a new wart, but reproduce normal skin (Perthes), showing that the stimulus to proliferation has been removed and that there remain epithelial cells capable of normal proliferating function.

12. The unknown factor in cancer is apparently added to normal epithelium, from which it can be removed, leaving normal epithelium. Through the proliferation of the cells of the cancer, which increase enormously, this factor must of necessity gradually increase in amount. The increase in bulk, through transplantation in mouse tumors, is associated with increased virulence. The only known agent which can fulfil these conditions is

a living organism. The unknown factor may be an ultramicroscopic organism, or one that is simply undemonstrable. Filtration experiments in infectious diseases of unknown etiology are not competent to throw any light on this phase of the subject.

13. Infectious venereal granuloma of the dog, an undoubtedly infectious tumor, presents certain points of similarity to malignant processes. The tumor grows largely through karyokinesis of the tumor cells which are derived from the connective-tissue cells of the host (Bashford). The cells do not appear to possess the power of limitless proliferation, although perhaps this is not conclusively proven.

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Conducted at the

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A study of the influence exerted by a variety of physical and chemical forces on the virulence of carcinoma in mice. By G. H. A. Clowes, Ph.D.

Evidences that infected cages are the source of spontaneous cancer developing among small caged animals. By H. R. Gaylord, M.D., and G. H. A. Clowes, Ph.D.

REPORT

January 9, 1907.

Dr. Eugene H. Porter, *Commissioner of Health, Albany, N. Y.:*

Dear Sir:— I have the honor to transmit herewith the Seventh Annual Report of the State Cancer Laboratory of the Department of Health.

As I communicated to you in the last annual report, the results of the work of the two preceding years enabled us to demonstrate that cancer is in principle a curable disease. We are now able to inform you that the work of this laboratory in that connection has been confirmed and amplified by publications during the past year, emanating from the laboratories of Professor Ehrlich, at Frankfurt, in Germany, the Royal Cancer Research Fund, of London, England, and from the Huntington Research Fund under the auspices of Cornell University, in New York.

A resumé of this work and the demonstration of the fact that this laboratory published the essential features of the recent advances in this field nearly a year in advance of all others will be found in an article by Doctor Clowes in this report. The particular advances in our own research during the past year are also set forth in this article. In the article on infected cages as the cause of cancer among small animals, we feel that the laboratory has been fortunate in obtaining evidence bearing directly on the contagiousness of cancer. The evidence in this matter I desire particularly to call to your attention, inasmuch as it bears directly upon the great economic question of the spread of cancer.

It has been customary since the foundation of the laboratory to incorporate in this portion of the report the number of deaths occurring in the current year from cancer. In reviewing the statistics from year to year it will be seen that cancer, since the foundation of this laboratory, has progressively increased. In order to compare the relative progress of tuberculosis and cancer we have determined the average death rate per 100,000 from these diseases, in the first place, for the years 1896, 1897, and 1898, collectively, and, in the second place, for the years 1903, 1904, and 1905, collectively, from which it will be seen that whilst tuberculosis has fallen from 186 per 100,000 to 177, cancer has increased from 59 to 74 per 100,000, representing for tuberculosis a decrease of 4.9 per cent., and for cancer an increase of 25.4 per cent.

	Tuberculosis.	Cancer.	Population.
1896	13,265	3,789	6,840,000
1897	12,641	4,131	6,988,000
1898	12,979	4,385	7,110,000
Average	12,961	4,101	6,979,333
Deaths per 100,000	186	59	
1903	13,194	5,456	7,614,281
1904	14,158	5,697	7,746,000
1905	14,061	6,056	7,918,000
Average	13,805	5,736	7,759,427
Deaths per 100,000	177	74	

While it is impossible to attribute the apparent increase in cancer entirely to the spread of the disease, there being other factors which readily cause an apparent increase, it must be conceded that in the light of the evidence pointing to the contagiousness of cancer, a great factor must be the absolute neglect of all precautions to combat the contagious element. The decrease in the spread of tuberculosis is entirely due to the recognition of its contagiousness. We have to-day no specific for tuberculosis. This change is due to the segregation of patients, fumigation of surroundings, sterilization of dressings, disposal of sputum, and a general and energetic campaign of education by the medical profession. In cancer, on the other hand, we find that nothing whatsoever is practiced, that many of the profession have been extremely skeptical as to its contagiousness, although the literature of the last few years has repeatedly contained articles showing that cancer has a tendency to occur endemically in certain localities, and that the occurrence of an unusual number of individual cases in certain houses is characteristic of the disease. How significant the evidence is, in favor of the surroundings of cancer patients becoming infected, is to be found in the ever-increasing number of publications directly bearing upon so-called "cancer-houses." It will be remembered that in the fourth annual report from this laboratory the results of a striking piece of research of a cancer district in western New York formed part of the report, and a further research in this line was incorporated in the sixth annual report of the laboratory.

During the past year a number of significant publications have appeared in the literature of cancer, and these, taken in conjunction with the striking cases of infected cages reported by others, and the remarkable occurrence which we ourselves present in this report, would indicate the desirability of the inauguration of a campaign against the spread of cancer similar to the successful propaganda against the spread of tuberculosis. I would suggest that the evidence is now sufficient for the Department of Health of the State of New York, as the result of researches conducted in this laboratory, to recommend to all health officers of the State the desirability of proper sterilization and disposal of all dressings of cancer cases, the fumigation and sterilization of rooms occupied by cancer patients. From the facts to be adduced from the infected cages under observation in this laboratory, it would appear that the contagion of cancer is extremely persistent, one cage having retained its infective characteristics for a period of not less than three years, a lapse of time equal to one-third the life of the animals which occupied the cage (white rats). For this reason, although there is little evidence that individuals associated with cancer cases are in any immediate danger of contracting the disease, it is extremely probable that the contagion of cancer can be transferred from cancer patients to their surroundings, where in course of time it assumes a form in which it is again capable of infecting susceptible individuals.

Since the inception of this laboratory the members of the staff have been greatly impressed with the possibilities of the parasitic theory of cancer, and while we have not neglected the various other lines of research, much of our work, especially our researches on immunity, has been more or less directed by this inclination. In the third, fourth, and fifth annual reports of this laboratory evidence was produced that cancer as a disease possessed certain points of comparison with other diseases, especially the acute exanthemata, syphilis, and possibly certain skin diseases. The peculiarities of the disease also suggested to us the probability that the organism of cancer was to be found either in the animal kingdom, or in that indefinite field which marks the boundary line between the lower animal and the vegetable world. Since the appearance of these reports a protozoan organism has been discovered in syphilis, and advances have also been made in the study of the suspicious structures found in the epithelial cells in smallpox. Following this line of research we have not neglected to systematically study the rich material which the laboratory now possesses, and we are now able to report that in all of our mouse cancers there is to be found, with striking regularity, an organism of the same species as the probable organism of syphilis. There is at present no positive proof that this organism is the cause of these tumors, but the regularity of its occurrence, and other lines of reasoning which are included in the article especially dealing with this subject, show that it is not beyond the bounds of possibility that future evidence may ultimately prove its etiological significance.

The organization of the staff of the laboratory remains practically the same as in our last report. It is greatly to be regretted that although, for the present year, the Legislature acceded to our request for a larger appropriation, the late Governor found it necessary, on the basis of a technicality, to cut from the appropriation $3,000 which was greatly needed. The next two or three years are sure to prove years of great activity in cancer research, which, as you know, is being prosecuted in several laboratories in Europe, and in at least two other quarters, under special appropriations, in this country. The New York State Cancer Laboratory is the first institution of its kind to be established in any country, and it is peculiarly appropriate that the State of New York should profit by the advantages which our early foundation has given us in this field. The amount of work which we shall be able to accomplish in the immediate future is largely dependent upon the amount of means at our disposal. Much of this present experimentation is simply a question of outlay for the necessary materials and sufficient assistance. We have now reached the point where we shall shortly desire to use larger animals than those we are employing, in order to attempt to apply the recently discovered facts to the ultimate cure of human cancer cases. As I pointed out to you in the last report, both the extensive laboratories of Professor Ehrlich and the Royal Cancer Research Fund, in London, enjoy larger resources than this laboratory, and for that reason I would ask that the item of $3,000, which was cut from the appropriation by the late Governor, be restored in the present appropriation, and that the total figure be fixed at $21,000. With this increase the scope of our work can be considerably enlarged.

In this connection I desire to call your attention to the fact that the principals in the laboratory have from the beginning served it with great devotion, on salaries which are not commensurate with their standing in the scientific world. The direction which cancer research has necessarily taken in the last two years necessitates the maintenance of a large staff of assistants, and with our present appropriation we are forced to employ a staff of thirteen individuals. The relative expenditure for stock, material, and equipment has so greatly increased by the direction which our work has necessarily taken, that the laboratory has from the first paid the lowest possible salaries to its employees. The great increase in the cost of living will make it necessary to remedy some of the greatest discrepancies in this connection during the coming year, unless the laboratory is to lose the services of some of its most important members.

The expenditures for the past year have been as follows:

September 30, 1905 —	Balance	$946	26
September 30, 1906 —	Stock and material	2,140	25
	Equipment	1,497	35
	Expense	2,151	29
	Salaries	11,012	98
	Total	$17,748	13

September 30, 1905 — Appropriation, 1905–6	$15,000 00
September 30, 1906 — Balance	2,748 13
Total	$17,748 13

The staff of the laboratory as at present constituted is as follows:

H. R. Gaylord	Director
G. H. A. Clowes	Chemist
G. N. Calkins	Biologist
F. W. Baeslack	Assistant in biology and histology
C. A. Maclay	Secretary
D. R. Averill	Assistant in photo-chemistry
F. A. Payne	Janitor

Six assistants classed as laborers.

I take pleasure in stating to you that we feel that the promises of the previous year have been amply fulfilled, that the work is assuming an ever-increasing interest and importance, and that after many years of tedious research the efforts of those who have supported the laboratory are being rewarded.

On behalf of the staff permit me to express our appreciation of the constant sympathy and support the laboratory has enjoyed from your Department. I am,

Very respectfully yours,
Harvey R. Gaylord, M.D.,
Director

On Spontaneous Cure of Cancer

By Harvey R. Gaylord, M.D., and George H. A. Clowes, Ph.D.

In view of the experiments conducted in this laboratory, which suggest the existence of immune forces antagonistic to cancer, it is deemed advisable to bring together the data regarding spontaneous cure in mice affected with carcinoma, and at the same time to see what evidence there is in the literature of spontaneous recovery occurring in human beings. However deep the skepticism may be regarding spontaneous cure of human carcinoma,—and this skepticism is certainly widespread,—there is absolutely no doubt of the occurrence of spontaneous cure in mice with cancer, no less than 101 clearly defined cases having come under our observation during the year just passed. The causes which lead to such cures, and the circumstances attending them, are all obviously of the first importance.

The question of spontaneous cure of cancer in human beings is of still greater interest and importance, and although but few authentic cases are to be found in the literature, they acquire an added significance when considered in conjunction with the results on animals recorded in this paper.

MOUSE TUMORS

Experiments on the transplantation of mouse tumors were commenced in this laboratory in the early part of the year 1904. In the autumn of the same year several tumors, after reaching appreciable dimensions, were found to be undergoing spontaneous retrogression, and eventually subsequent examination showed that they had entirely disappeared. Since that time recoveries from true tumors have occurred repeatedly, and we purpose at this stage to present the evidence thus far accumulated regarding the conditions under which these spontaneous recoveries are effected and the frequency of their occurrence. Very few references to spontaneous recoveries are to be found in the rapidly growing literature dealing with the transplantation of mouse tumors.[1]

Michaelis (*Verhandlungen des Komitees f. Krebsforschung,* h. iii, 1903–1904, p. 38), in discussing the results obtained by inoculating Berlin mice with the Jensen strain, mentions the occurrence of tumors which, after reaching the size of a pea, remained stationary, and subsequently retrogressed and entirely disappeared.

Apolant (*Deut. med. Wochenschr.*, 1904, No. 13), in a paper dealing with the influence of radium on mouse tumors, refers to cases of spontaneous recovery, two of which are actually illustrated in the series of control mice. One of these had reached a diameter of four millimeters, slightly above the minimum which we have employed. The fact that Apolant considers that there is no risk of mistaking spontaneous cures for those affected by the radium rays, when working with tumors which have already reached considerable dimensions, makes it apparent that spontaneous recoveries from large tumors had not, up to that time, been observed in Ehrlich's laboratory. Bashford, in his last report, states that he has observed in some 3,000 tumors but one case of spontaneous recovery of a tumor having a diameter of one centimeter.[1] We ourselves had not, previous to the publication of our first communication regarding the activity of the serum of spontaneously recovered mice, observed any cases in which tumors over five millimeters in diameter recovered spontaneously.[2]

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[1] The first accurate observation of retrogression of experimentally inoculated tumors was made by Wehr (*Centralbl. f. Chir.*, vol. xv, 1888). This author, in 1883, inoculated a series of dogs subcutaneously with fragments of a medullary carcinoma taken from the vaginal mucosa of a bitch. He used for this purpose small fragments of tumor removed aseptically. Inoculation was not followed by inflammatory reaction. There was during the first eight days no swelling in the region of inoculation, neither could the inoculated fragments be palpated. Later there appeared nodules which grew to the size of beans or hazelnuts in the course of six to eight weeks, after which they remained for some time stationary and then gradually disappeared. A number were removed for microscopical examination at various stages, and showed that the tumors were composed of carcinoma tissue of identical structure with the original tumor. In all of the sections examined he was able to demonstrate in the epithelial cells of the tumors frequent karyokinetic figures. L. Loeb (*Virchow's Archiv*, bd. 167, h. 2, 1902) in the transplantation of sarcoma in rats, observed that in many cases tumors remained stationary and ultimately retrograded. Sticker (*Zeitschr f. Krebsforschung* vol. i, No. 5, 1904), also, in the course of transplantation of a round-celled sarcoma in the dog, observed spontaneous retrogression of tumors of considerable size.

In a previous communication the methods of transplantation have been dealt with in detail. We, therefore, refer to them only in so far as is necessary to demonstrate what attempts have been made to render our results strictly comparable from a biophysical standpoint.

With a few exceptions, tumors which have been used for transplantation were carefully weighed and ground in a mortar with either three or four volumes of physiological salt solution (.8 per cent.). All large aggregates of tissue which might interfere with the uniformity of the dosage were then removed by means of fine rakes. In all series of experiments in which a comparison was made between the influence exerted by varying sera, chemical bodies, or temperatures on the materials previous to injection, equal volumes were separated into uniform tubes, the greatest precaution being observed to obtain a perfect admixture of the materials before measuring the individual portions. Subsequently, each individual mouse in a series received a subcutaneous injection of exactly the same volume, usually about half a cubic centimeter of the macerated cancer mixture.

Up to the present date (January 1st) about 3,500 mice have been employed in this laboratory for the experimental transplantation of a series of tumors. Of this number, by far the larger part — over 2,500 — have received injections of materials derived from the Jensen tumor. Upward of 550 fatal tumors have been obtained in this series, and, in addition, over 300 mice have shown, at some stage, indications of a possible tumor development. In this latter series 101 cases of undoubted spontaneous recovery from true tumors which had reached such a size and age as to exclude the possibility of experimental error have been collected, and form the basis of the present communication.[1]

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[1] Bashford (Scientific Reports on the Investigations of the Imperial Cancer Research Fund, No. 2, pt. ii, 1905) apparently makes use of small portions of tumor introduced by means of a trocar. He makes no mention of the employment of weighed quantities of material, and considering the enormous variation in the composition of any one tumor in various parts it is evident that even were uniform weights of material employed, the results, from a statistical standpoint, would be in no sense comparable.

[2] Medical News, January 14, 1905.

That we have been able to tabulate these results and demonstrate the occurrence of such a large proportion of spontaneous recoveries is owing to the fact that we very early adopted a system of identifying the individual members of an experimental series and charting each mouse at intervals of five or six days from the date of original inoculation. For this purpose chart-books are employed, in which the groups are arranged numerically and the individual members of a group alphabetically, and for each individual a series of diagrams is prepared, showing the fore legs, hind legs, nose, and tail. When a tumor makes its appearance on the back of a mouse it is reproduced in the diagram as nearly life-size as possible and in the location in which it occurs, and as the tumor increases in size and secondary tumors are produced, further chartings are prepared, showing the progress of the tumor growth until the death of the animal occurs.

Upward of 1,800 mice have been inoculated with the Jensen strain since the adoption of this system, and 711 mice have actually been charted for a shorter or longer space of time. These were distributed as follows: Four hundred and forty-five fatal tumors, 400 of which reached considerable dimensions, the remainder being present on animals that succumbed before the tumor had reached any considerable size. There were, in addition, 101 undoubted spontaneous recoveries referred to above. The remaining chartings were cases of questionable spontaneous recovery or those in which the tumor materials ulcerated.

The most remarkable differences in the speed of development of tumors, even in the same series, occur continually. Some tumors have developed so slowly as to permit of the mice having been charted from twenty-five to thirty times, at intervals of six to eight days. Others have developed so rapidly that two or three records of chartings are all that are to be found previous to the death of the animal. By examining these charted records it may be seen at a glance that a large number of the tumors developing in the usual manner in from two to three weeks from the date of inoculation have gradually increased in size until they have reached considerable dimensions, and have then, after remaining stationary for a while, begun to diminish in size, eventually disappearing entirely. In order to exclude all early inflammatory processes which, occurring within a few days after inoculation, may occasionally be mistaken for true tumors, we have, in a previous publication, adopted for a standard of spontaneous recovery the existence of a growing tumor not less than twenty days from the date of inoculation, reaching a maximum of not less than three and a half millimeters in diameter at some stage of its development. ("Further Evidence of Immunity against Cancer in Mice after Spontaneous Recovery," *Medical News,* November 18, 1905.) Several of these spontaneous recoveries have been intercepted at a stage at which no doubt could exist that retrogression had set in. The tumors, when removed, afforded ample pathological evidence of recovery, the details of which will be given later.[1]

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[1] Upwards of six hundred mice have been used for the transplantation of a sporadic tumor obtained in this country. A considerable number of spontaneous recoveries have been obtained in this series, but we have not included the results in this paper, owing to the short time which has elapsed since the commencement of work on this series.

We have every reason to believe that the increased number of spontaneous recoveries in our records of the last twelve months, during which we have employed our present charting system, is attributable rather to the improved method of laboratory observations than to any marked falling off in the virulence of the tumor materials, which have, in spite of intermittent variation, maintained a fairly uniform average. It is interesting to note that whilst only one case of spontaneous recovery from a tumor over five millimeters in diameter was noted in the 105 actual tumors obtained from the first 450 mice inoculated, the apparent yield of spontaneous recoveries has risen since the adoption of the charting system to from 18 to 22 per cent. The number of tumors which after reaching a diameter of five millimeters retrogress spontaneously is found to be sixty-six; about two-thirds of the total number recorded above, and over 12 per cent. of the total number of true tumors which made their appearance during this period. Only eleven tumors are found to have reached a diameter of one centimeter or more before retrogression; rather more than 2 per cent. of the total number of tumors. This figure is still considerably in excess of that given by Bashford, who observed only one case in 3,000.

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[1] Bashford, in his report (page 62, footnote), intimates that our large percentage of spontaneous recoveries might be accounted for by assuming that we had mistaken early inflammatory swellings in the subcutaneous tissue attributable to septic processes for true tumors. We would point out that, simultaneously with the publication of Bashford's report, reference was made in a communication from this laboratory to these early inflammatory swellings, and a provisional standard for the recognition of a true tumor adopted, as follows: "The presence of a growing tumor not less than three weeks after inoculation, reaching at some period of its development a volume of over twenty cubic millimeters."

By measuring the tumors approximately a very fair degree of accuracy may be obtained in the charting process, especially during the early stages of development. Whilst the tumors are still small they are, generally speaking, spherical or ovoid in form, and consequently a very fair estimate of the actual cubic content of such a tumor at any stage of its development may be obtained from the diagrams by means of the formula, $d_1 d_2 d_3 \frac{\pi}{6}$, in which the third diameter is assumed to be approximately equal to the smaller of those actually recorded. We have adopted this method of computation in order to estimate the relative speed of development of the tumors of different groups, and also with a view to comparing the progress of those tumors which result fatally with those from which the mice recover spontaneously. It must, however, be borne in mind that such a method of estimating the total cubic content, whilst relatively accurate in dealing with small tumors, is liable to error as the size of the tumor increases, owing to the tendency of the majority of tumors to flatten out over an area, the width of which is considerably greater than the thickness. It should, therefore, be distinctly understood that in subsequent portions of this paper, where reference is made to the volume of a tumor, it is merely an approximate estimation of the volume in question, and simply employed for comparative purposes, without any assumption that the results exhibit the absolute values; however, for the sake of control, we have frequently made comparisons between the weights of the tumors at autopsy and their volumes as estimated from the dimensions when last charted, and the results have led us to believe that our method of computation affords reasonably accurate results.

After carefully eliminating all those cases to which any possible objection could be raised, we find 100 undoubted spontaneous recoveries occurring in the course of one year from the commencement of the charting system. On account of space limitations it would be impossible to give full details regarding each of these cases. We have, therefore, prepared tables showing the relative distribution according to maximum dimensions reached by the tumors, also showing the relative distribution according to the period of time elapsing between the dates of inoculation and first appearance, and those of first appearance and maximum size. A few individual cases will be considered separately at a later stage.

I.[1] Distribution of tumors according to maximum dimensions.[2]

Volume of Tumor, in c.m.	Number of Cases	
50 and under	25	46
51 to 100	21	
101 to 150	18	33
151 to 200	15	
201 to 250	6	11
251 to 300	5	
301 to 350	1	2
351 to 400	1	

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[1] In this and succeeding tables, as stated above, all tumors which failed to reach a minimum volume of twenty cubic millimeters, not less than twenty days after inoculation, have been excluded.

[2] It is interesting to note that the average maximum size reached by these cases of spontaneous recovery is over 200 cubic millimeters, or representing approximately a diameter of 7⅓ millimeters.

Volume of Tumor, in c.m.	Number of Cases.	
401 to 450	0	1
451 to 500	1	
Total under 500	93	
501 to 1000	2	
Total under 1000	95	
1001 to 2000	3	
2001 to 3000	1	
Over 3000	1	
Total	100	

An inspection of this table shows a steady decrease in the number of spontaneous recoveries, with increasing maximum volume reached by the tumor. In other words, the greater the dimensions reached by a tumor, the smaller are its chances of spontaneous recovery. Ninety-five per cent. of the recoveries failed to reach a volume of 1 c.c., 3 have a volume lying between 1 and 2 c.c., and only 2 above that point. This fact is extremely significant when taken in conjunction with those enumerated in a preceding portion of this paper dealing with human cases. In the case of our experimental mice, having absolute control of our materials and observing the greatest precautions in following the progress of inoculated individuals from day to day, we are obviously able to detect the presence of extremely small tumors at a much earlier stage than would be possible were we dealing with human subjects; and seeing that the chances of spontaneous recovery apparently decrease in a direct ratio to the increase in size of the tumor, it is fair to suppose that cases of early spontaneous recovery in human beings frequently escape observation.

For the sake of comparison of the speed of development of tumors which recover spontaneously with others which result fatally, we have prepared two tables, as follows:

II. Showing the distribution of 100 spontaneous recoveries, according to period of time elapsing between date of inoculation of mouse and date of first appearance of tumor.

Period, in Days	Number of Cases
10 and under	8
11 to 20	39
21 to 30	27
31 to 40	13
41 to 50	4
Total under 50	91
51 to 100	6
101 to 150	2
151 to 200	1
Total	100

III. Distribution of 337 fatal tumors, according to period of time elapsing between date of inoculation of mouse and date of first appearance of tumor.

Period, in days	Number of cases.	Percentage
10 and under	36	10.7
11 to 20	112	33.2
21 to 30	94	27.9
31 to 40	35	10.4
41 to 50	23	6.8
Total under 50	300	89.0
51 to 100	32	9.5
101 to 150	4	1.2
151 to 200	1	.3
Total	337	100.0

These two series represent the spontaneous recoveries and fatal tumors occurring in the same period, and are, therefore, strictly comparable, and it will be seen at a glance that the figures obtained correspond very closely. In each case the largest percentage of first appearances occurs in the period lying between 11 and 20 days from the date of inoculation, followed by that between 21 and 30 days. From this point there is a gradual fall, only a few isolated cases appearing in each series after 100 days from the date of inoculation. It must, therefore, be said that, so far as the first appearance of the tumor is concerned, we have no indication of any difference existing between the speed of development of those tumors which result fatally and those which recover spontaneously.

IV. Distribution of 100 spontaneous recoveries, according to period of time elapsing between date of inoculation of mouse and date on which tumor reached maximum dimensions previous to commencement of retrogression.

Period, in Days.	Number of cases.
21 to 30	35
31 to 40	27
41 to 50	18
51 to 60	8
61 to 70	4
71 to 80	2
81 to 90	1
91 to 100	2
Total in 100	97
101 to 150	2
151 to 200	1
Total	100

From this table it will be seen that more frequently spontaneous recoveries occur in the earlier periods; a conclusion which is only to be expected if the findings in table I, dealing with the maximum dimensions reached, are correct. The two tables, taken in conjunction, make it probable that the farther the tumor has advanced, that is to say, the longer it has been growing and the greater its size, the smaller are its chances of retrogressing spontaneously.

In addition to the above tables, others have been prepared, showing the distribution of spontaneous recoveries and fatal tumors, according to the periods of time elapsing between dates of inoculation, dates of first appearance, and dates on which tumors reached certain dimensions. Some of these tables have been omitted in order to economize space, but it may be stated briefly that practically no difference exists between the distribution of spontaneous recoveries and fatal tumors with reference to the period elapsing between date of inoculation and date on which a diameter of six millimeters was reached. On the other hand, it would appear from the figures recorded below, showing the distribution of forty spontaneous recoveries and one hundred comparable fatal cases with reference to the period of time elapsing between the first appearance and a diameter of six millimeters, that the development of the fatal tumors is rather more rapid than that of the spontaneous recoveries.

Period, in Days.	Percentage of Spontaneous Recoveries.	Percentage of Fatal Tumors.
10 and under	35.0	56.0
11 to 20	40.0	24.0
21 to 30	22.5	15.0
31 to 40	0.0	3.0
41 to 50	2.5	1.0
51 and over	0.0	1.0

We are not prepared, however, to attach much importance to these figures until they have been confirmed by a much larger series of experiments than has up to the present been found possible. The figures in question are more subject to experimental error than is the case with any other determinations referred to above, seeing that two relatively uncertain dates have to be determined — that of first appearance and that on which a given volume was reached.

In connection with this rather questionable evidence that the chances of recovery are smaller the greater the speed of development of the tumor, it is interesting to note that when several tumors develop in a given series, some rapidly and others slowly, the yield obtained on transplantation from the more rapidly growing tumors is usually higher than that obtained from the more slowly developing members of the series.[1] The two above facts taken in conjunction would indicate the probability that the more rapid the development of a tumor the greater is its virulence, and consequently the greater must be the natural immunity possessed by the animal in order that a spontaneous cure may be effected.

To summarize the results of the preceding statistical section, it may be said that the chances of spontaneous recovery diminish with increasing age and dimensions reached by the tumor, the percentage of recoveries falling from 21.7 per cent. in the tumors that have not yet reached a volume of 1 c.c to 1.1 per cent. in those which have exceeded that size. There is also a certain amount of evidence that the chances of recovery are greater the slower the growth of the tumor, but this point is one on which the statistics are to a certain extent conflicting, and on which further evidence must be obtained before it can be finally accepted.

INDIVIDUAL CASES OF SPONTANEOUS RECOVERY

For the sake of demonstrating the course followed by tumors which have recovered spontaneously, we have included in this report the following charted records.

Above each series is found the number and letter of the mouse and over each diagram the date on which the charted record was made. (See Plates I and II.)

The first case, 217A (see Plate I, Diagram 1), a mouse weighing 22½ grams, had the largest tumor so far recorded as recovering spontaneously. It may be roughly estimated to have reached a volume of over three cubic centimeters at its maximum point, and if one considers a cubic centimeter of tumor material as approximately equal to a gram, it will be seen that the tumor actually represented about two-fifteenths of the entire weight of the mouse.

Inoculated March 28, 1905, the tumor made its appearance on April 17th, a small spherical mass 3 mm. in diameter being observed in the loose tissue above the right shoulder. This rapidly increased until, on April 27th, a clover-leaf-shaped aggregate made its appearance, which in all probability, resulted from the development of a second focus of growth and the prolon-

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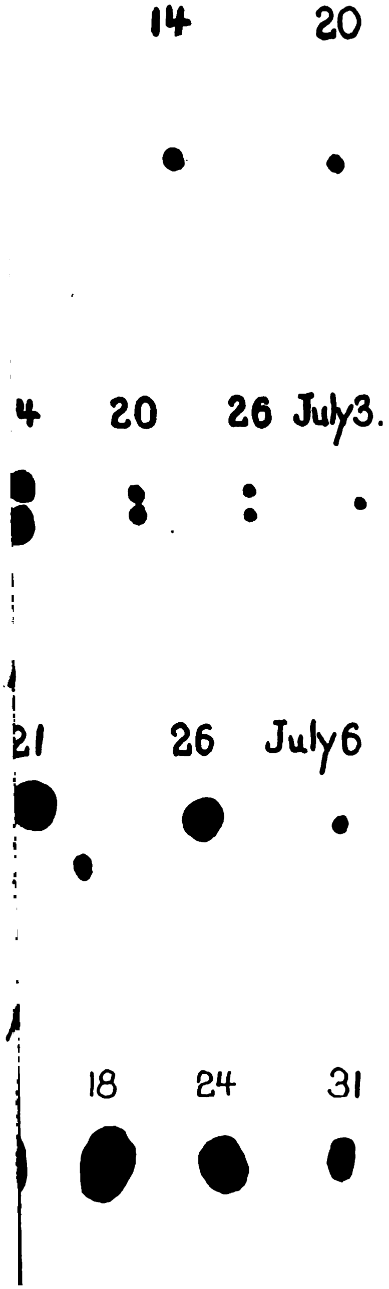
[1] Journal of Experimental Medicine, July, 1906.

Plate I.



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gation of the first tumor to the left. On the 3d of May a large mass was present on the animal's back, extending from the back of the head, and measuring in its maximum dimensions 25 mm. On May 10th a change was to be observed in the size and shape of the tumor, which still retained a form indicative of the existence of two distinct tumors. On May 17th, only seven days later, a great change was noticeable. The upper tumor had now diminished until its diameter was under 10 mm., whilst the lower tumor had fallen to 6. On the 25th of May the tumors had still further diminished in size, and might now be readily distinguished from one another. On June 2d the lower tumor had entirely disappeared. On subsequent dates the remaining tumor rapidly diminished in size, having a diameter of only a millimeter on the 20th of June, and probably consisting of a small grain of connective tissue; and finally on the 26th of June no tumor at all was to be observed. The mouse was examined repeatedly without any indications of further tumor development being discovered, and a subsequent inoculation on September 28th with virulent materials capable of producing tumors in other normal series failed to produce any effect upon this animal.

215C (see Plate I, Diagram 2) is another remarkable illustration of this type of spontaneous recovery from a large tumor. 215C was inoculated on the same date with similar materials and similar dosage to those which were employed for 217A. The tumor made its appearance on the same date on which the tumor was first observed in 217A, and increased at about the same speed, reaching its maximum between May 17th and May 25th, subsequently diminishing in size, and finally entirely disappearing about the end of July. The subsequent inoculation of this mouse failed, as in the case of 217A, to produce a second tumor, both animals being apparently possessed of a very powerful acquired immunity.

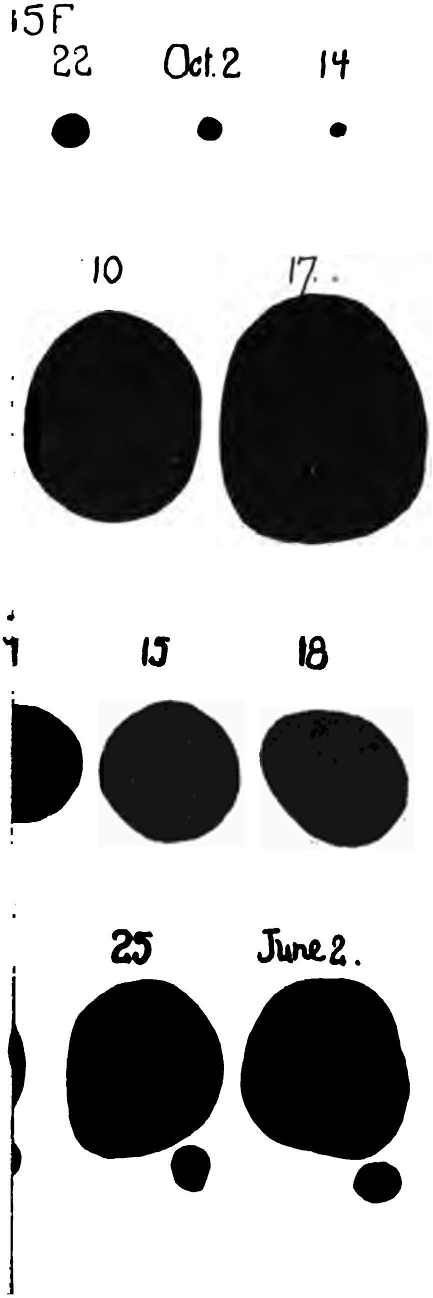
212A and 213D (see Plate I), two other members of the same series as those which have been referred to, showed smaller tumors, disappearing in a shorter space of time, but the fact that they were in both cases increasing in size twenty days after inoculation, and reached dimensions of 12 mm. and 8 mm. diameter respectively, considerably above the minimum of 3½ which we have adopted, has led us to include them with many others of the same type in the series of spontaneous recoveries, the tabulated results of which have been referred to above. An examination of the charts shows in both cases a uniform rise to a maximum, and a subsequent fall and final disappearance. It may also be noticed that in each case, on the date on which the main tumor had reached its maximum dimensions, a secondary small tumor had made its appearance, and subsequently disappeared previous to the entire subsidence of the larger tumor. In fact, in the great majority of cases two or more tumors make their appearance at some stage in development, and the speed at which they disappear is usually proportional to their size.

It is remarkable that two out of four of the largest tumors which retrogressed were in the one series 212 to 219, inoculated on March 28th from 175C, but only 7 out of the 100 spontaneous recoveries tabulated above occurred in this group. The total number of true tumors which made their appearance in this series was 30. The proportion of recoveries, 7 in 30, or 23⅓ per cent., is not, therefore, much in excess of the normal ratio of spontaneous recoveries observed in the entire series.

304C, the chartings of which have also been reproduced (see Plate I), comes in an entirely different series from those dealt with above. Inoculated April 26th from 200A, a very virulent strain, it showed no tumor as late as May 10th, but on May 17th a spherical mass 6 mm. in diameter had made its appearance in the back of the neck. This increased rapidly, until on the 31st of May its diameter was 13 mm. In the meantime a secondary small tumor had made its appearance on the right side of the abdomen. From the 31st of May onward both tumors gradually decreased in size, until on June 26th the smaller had entirely disappeared, and finally, on July 14th, no trace of any tumor was left, and further inoculation of this mouse on a subsequent date failed to give a second tumor.

We realize that in those cases in which we allowed the tumor to recover completely, we have no absolute evidence that a true tumor ever existed. When compared, however, with the course of development of those tumors which eventually cause the death of the animals, there can be no doubt in the mind of the observer that we are dealing with true tumors. In order to test the accuracy of our clinical observations, we have at random chosen a certain number of tumors which were in obvious course of retrogression, and extirpated them for histological examination. In all of these cases the histological evidence was conclusive. Final judgment

Plate II.



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must be based on a combination of these two methods of procedure. The tumors selected for removal and histological examination were 335F, 338A, 421B, and 513F, diagrams of three of which have been included in this report.

335F (see Plate II), a slow-growing tumor, had reached a maximum of 10 mm. 112 days after inoculation, when it began to decrease, and in the course of 36 days fell to a diameter of 2 mm., when it was removed for examination.

Hardened in sublimate, stained with hæmatoxylin-eosin and Borrel. Examination with low power shows the tumor to be surrounded by a well-defined capsule of fibrous connective tissue. One portion of this capsule contains a certain amount of fat tissue and some large capillaries. An inspection of the margin of the tumor shows a well-defined round-celled infiltration, with frequent small capillaries. The structure of the tumor presents in the center the usual characteristics of a tumor of this age, and consists of large alveoli, the margins of which are lined by a cell-complex four or five rows deep. Generally speaking, the centers of these alveoli contain necrotic material. The stroma is well-defined, and shows marked evidence of round-celled infiltration throughout. The epithelial cells present the usual irregularity of outline, deeply stained protoplasm, and vesicular nuclei of this type of mouse tumor. Inspection of the margin of the tumor shows a great variation in the thickness of the capsule. In one aspect of the tumor it is a thin, delicate, but still well-defined structure, its outermost layers consisting of fibrous connective tissue, then a layer of fine fibroblasts intermixed with round cells. At this point the complex of tumor-cells rests directly upon the delimiting capsule. The remaining circumference of the capsule shows, however, extensive round-celled proliferation, and here and there areas of well-defined granulation tissue. At one of the poles of the tumor this granulation tissue is very extensive. It has penetrated into the larger alveoli, and we find small nests of epithelium widely separated from each other by areas of proliferating connective tissue. Here and there we find remnants of the necrotic material from the center of the original alveoli of the tumor. Viewed with low power, the epithelial cells immediately adjacent to the connective tissue in those areas where the nests have been split up appear, as a rule, to be smaller and more deeply stained than the epithelial cells in the centers of the nests. The connective tissue has a plentiful supply of capillaries, and small areas of hemorrhage are frequent. Under high power the margin of the tumor is of the greatest interest. Everywhere we find active proliferation of connective tissue, strands of which penetrate between the epithelial cells. Round-celled infiltration at the margin is very well defined. Where the epithelium is split up into small nests, the cells at the margins of these nests are atrophic. and the chromatin of the nucleus is uniformly diffused through its substance, the nuclei are small and deeply stained; the protoplasm is not stained as deeply as that of the cells at the center of the nest and is scanty. Occasionally an isolated epithelial cell is found detached near the margin, in which case the nucleus takes the nuclear stain but faintly, the protoplasm has nearly disappeared. Occasional cells are also found about the margins in which the nuclei present characteristic karyolytic changes, the cell presenting the appearance of a remnant of protoplasm with chromatin irregularly distributed through it. Proceeding from the margin to the center of these nests, one finds that the cell boundaries of groups of cells near the margin are frequently indistinct. Here and there a group of several epithelial cells have coalesced, forming the characteristic pseudo giant cells of Becher and Petersen. The cells at the center of these nests are indistinguishable from the characteristic epithelium of the growing portions of this tumor. Karyokinetic figures are occasionally met with in the cells at the center of the nests. Rarely they may be found nearer the margin, but never at the extreme margin. In sections stained with Borrel's method, the connective-tissue nuclei are deep red. The epithelium is easily distinguished from the connective tissue by the orange protoplasm and pale red vesicular nuclei. In sections so stained, the isolated epithelial cells may be readily detected and easily distinguished from the surrounding connective tissue. They are small, the nucleus stains poorly, the protoplasm has nearly disappeared. Occasionally, groups of two or three, forming a pseudo giant cell, are to be observed. Careful search of this preparation fails to show the presence of any typical foreign-body giant cells.

338A (see Plate II, Diagram 1) was a slowly developing tumor which grew to a maximum of 9 mm. in diameter by the eighty-fourth day, when it began to diminish in size, until on the one hundred and thirty-second day it measured 4 x 3 mm., when it was removed, hardened in sublimate, sectioned, and stained with hæmatoxylin-eosin and Borrel.

The tumor is surrounded by a dense capsule, thickest at the poles of the tumor. The area included in connective tissue may be said to be approximately one-half of the mass. The centers of the alveoli of this tumor contain extensive areas of typical necrotic material. The alveoli of this tumor are large. One aspect of the tumor is a portion of the skin of the animal, beneath which are strands of muscle, and then the connective tissue surrounding the tumor. There are evidences at the margin that the tumor originally pentrated into the muscle, and we find atrophic muscle fibers imbedded in connective tissue. The outermost layer of the capsule consists of an extensive layer of fibrous connective tissue, in which are groups of cells laden with hæmatogenous pigment. Occasionally remnants of a nest may still be found immediately within this dense portion of the capsule, and in some cases these are surrounded by aggregations of pigmented cells. The changes at the margin of this tumor may be divided into two classes; viz., isolated nests of retrograding epithelium, and isolated masses of necrotic material. The masses of necrotic material lie nearer the periphery of the capsule than do the isolated nests of epithelium, and appear to be the remnants of larger alveoli from which the epithelium has disappeared, leaving the necrotic material imbedded in the connective tissue. The capillaries in the capsule of this tumor are plentiful, and areas of hemorrhage directly beneath the epithelium, especially into the crevices separating the epithelium from the

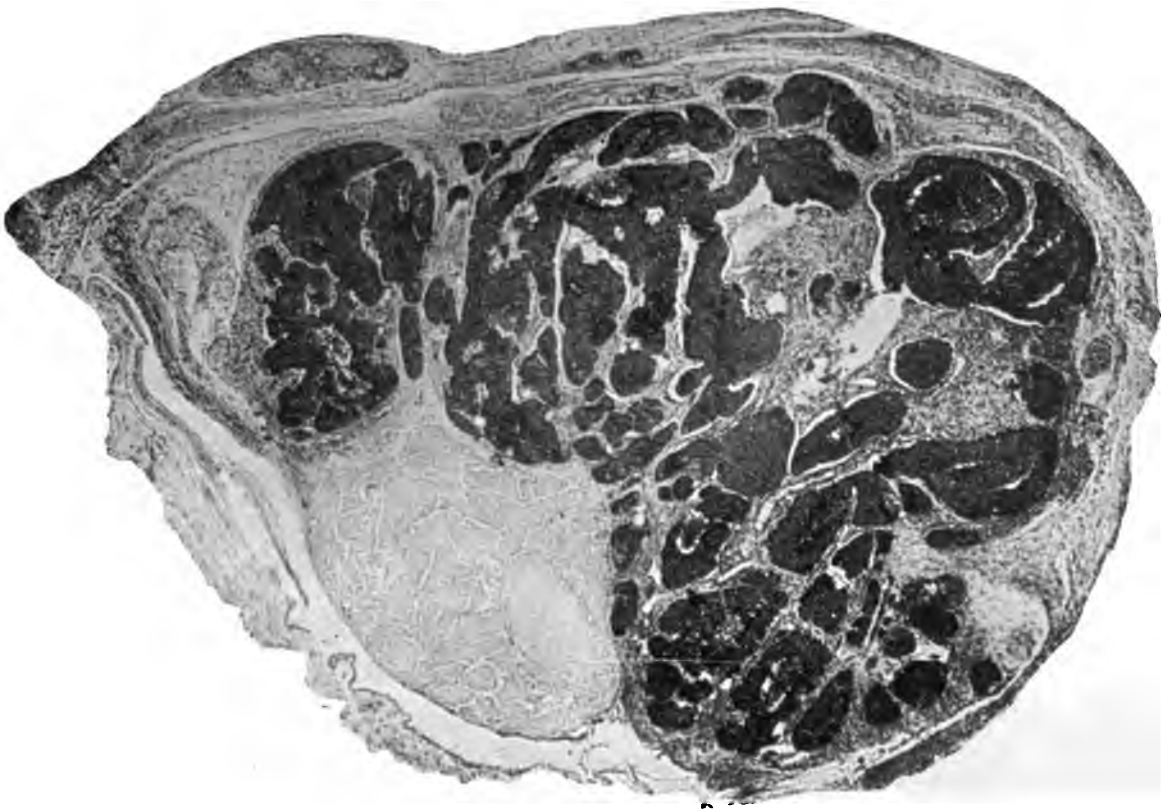

Fig. A. Low power. Complete section through rapidly growing implanted tumor. Below and to the left, hyaline detritus, remnants of injected material. It is to be noted that the structure grows in a radiating fashion from this mass Micro-collinear.

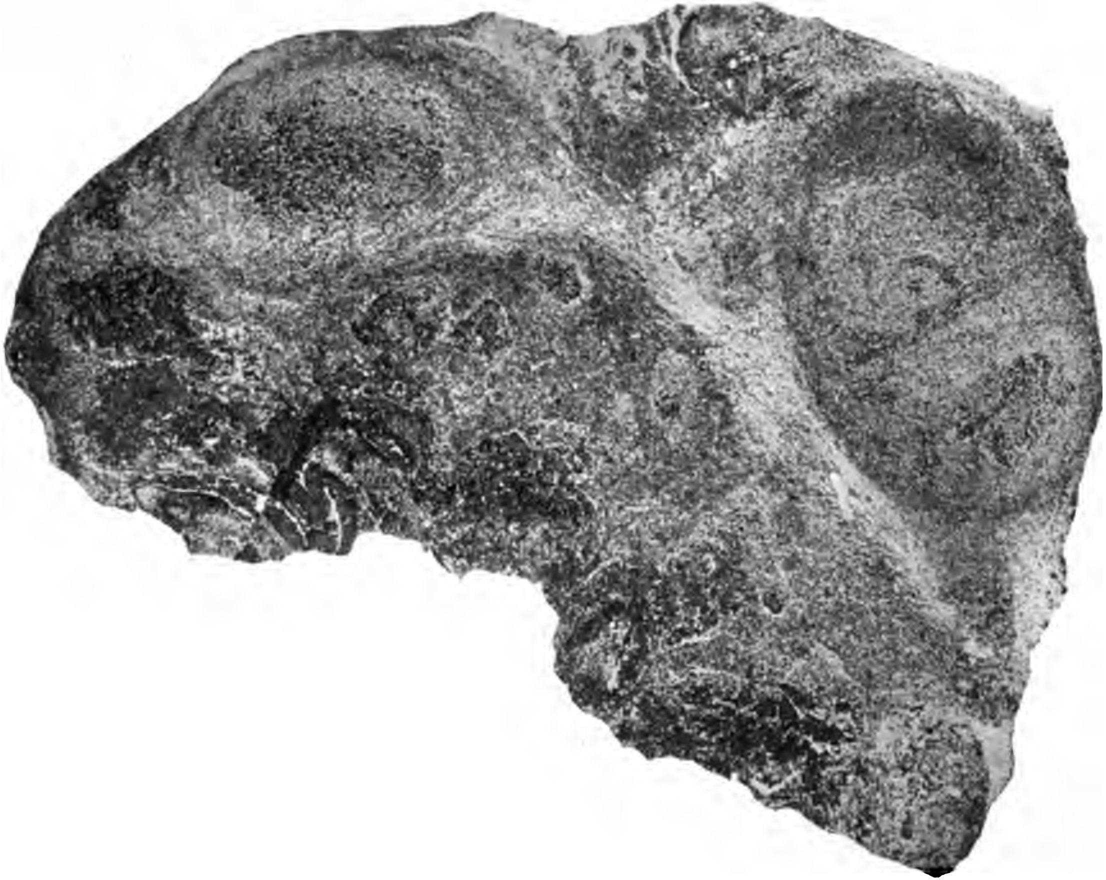

Fig. B. Margin of retrograding tumor. Below and to the left, growing epithelium presenting characteristics of that seen in Fig. A. Both right and left, areas of connective tissue marking [illegible] with remnants of epithelium in the connective tissue. Micro-collinear.

connective tissue of the stroma, are frequent. From these hemorrhages we may ascertain the source of the brown pigment in the cells of the older portions of the capsule. An examination of the epithelial nests at the margins of the tumor show the characteristic penetration of the connective tissue between the epithelial cells, splitting them up into smaller isolated nests. The marginal cells of these nests show the characteristic atrophy, the coalescence of groups of cells into pseudo giant cells, and at the center occasional karyokinetic figures. An examination of the areas of necrotic material imbedded in the connective tissue capsule shows a totally different picture. Here we have at the margins of these masses numerous foreign-body giant cells. These giant cells frequently contain large numbers of nuclei, arranged in the characteristic manner at the margins of the cell or closely grouped at the center. The nuclei stain diffusely and deeply. The protoplasm takes the eosin well, and merges gradually into the protoplasm of the adjacent connective-tissue cells. These giant cells are very plentiful; as many as thirty or forty can be counted about the margins of some of the masses of necrotic material. The connective tissue immediately adjacent to these remnants of necrotic material is of distinctly fibrous character, whereas that surrounding the isolated nests of epithelium is of the younger variety. Under high power an examination of the margins of some of the larger nests discloses the areas of hemorrhage above referred to. Here one sees the red-blood corpuscles separating the connective-tissue structure and extending in many cases between the epithelial cells. The epithelial cells at the margins of these nests where hemorrhage has occurred show very marked atrophic changes. An examination of the relation of these areas of hemorrhage to the epithelium strongly suggests that there is some connection between the extravasation of blood between the cells and the marked atrophy. Examination of the outer portions of the capsule show that the brown pigment is deposited in large spindle and occasionally branching connective-tissue cells.

421B reached its maximum 49 days after inoculation (see diagram), when it measured 7 mm. in diameter, then decreased in size until, 14 days later, it was removed for examination. This tumor was not allowed to retrogress to the extent of those already described.

The tumor is surrounded by a dense mass of connective tissue, in which are imbedded occasional bundles of muscle, indicating that it originally penetrated into the muscular structure. Large areas of connective tissue are found, containing only occasional groups of epithelium. In one portion we have what was evidently a large alveolus in the epithelium, split up into small groups of cells, many of which have coalesced, forming typical pseudo giant cells with connective-tissue fibroblasts, round cells, and occasional capillaries through the entire structure. Isolated nests of epithelium imbedded in the connective tissue are met with everywhere. In the epithelium are occasional karyokinetic figures, and at the center of the tumor characteristic alveoli lined with thick layers of epithelium. The connective tissue surrounding the tumor is at least equal in bulk to the remaining epithelial structure. The whole presents the characteristic picture of a tumor undergoing rapid retrogression. There are no isolated masses of necrotic material or foreign-body giant cells.

Tumor 161F (no diagram) is of interest, in that it was the first spontaneous recovery removed for histological examination. It occurred before the present charting system, and we have no knowledge of the exact maximum size of the tumor, but it was about that of a pea. It shows the most advanced stage of retrogression of any of the tumors here described.

This tumor consists of a small nodule about the size of a small grain of rice. Under low power it is found to consist of a connective-tissue structure, at the margins of which are capillaries, the whole surrounded by a delicate, fibrous connective-tissue capsule. Near the center of this mass of young connective tissue are groups of widely separated epithelial cells. In many cases these form aggregates of coalesced cells, presenting the appearance of pseudo giant cells. In many sections there are but one or two groups of these pseudo giant cells. In the sections representing the center of the tumor, one finds somewhat larger aggregates of cells, which may be spoken of as isolated nests. Examination of these cells with high power serves to define clearly their nature. They are epithelial cells with characteristic vesicular nuclei and deeply staining protoplasm. In almost every case the cells are broken up into groups of six or eight, the protoplasm of which has coalesced. Occasionally, markedly atrophic cells are seen, sometimes the remnants of pseudo giant cells with one or two nuclei, or occasionally single cells with poorly staining nuclei and scanty protoplasm. In the larger nests there is a distinct suggestion of the original arrangement of the epithelium. There is no doubt that these cells represent the last traces of what was previously a well-defined alveolar structure lined with the characteristic epithelium of the Jensen tumor.

513F (see Plate I), a mouse, which had previously been inoculated unsuccessfully, received an injection for the second time on the 25th of October. A tumor made its appearance on the 11th of November. This tumor gradually increased in size until a maximum of 12 x 15 mm. diameter was reached between December 18th and December 28th. From this point the tumor gradually diminished in size until February 1st, on which date, 98 days after inoculation, a residue measuring 6 x 3 mm. was removed for examination.

Examination of the section (stained with hæmatoxylin-eosin) with low power shows a mass of connective tissue, in which are several large blood-vessels. In the center of this mass are areas of necrotic material surrounded by a wide zone of connective tissue. Under high power the necrotic material presents the irregular outlines of the characteristic masses of necrotic cells in the center of alveoli of the Jensen mouse tumor. In a section stained with Borrel, the masses of necrotic cells are stained green, the surrounding connective tissue blue. This necrotic material is distributed through the tissue

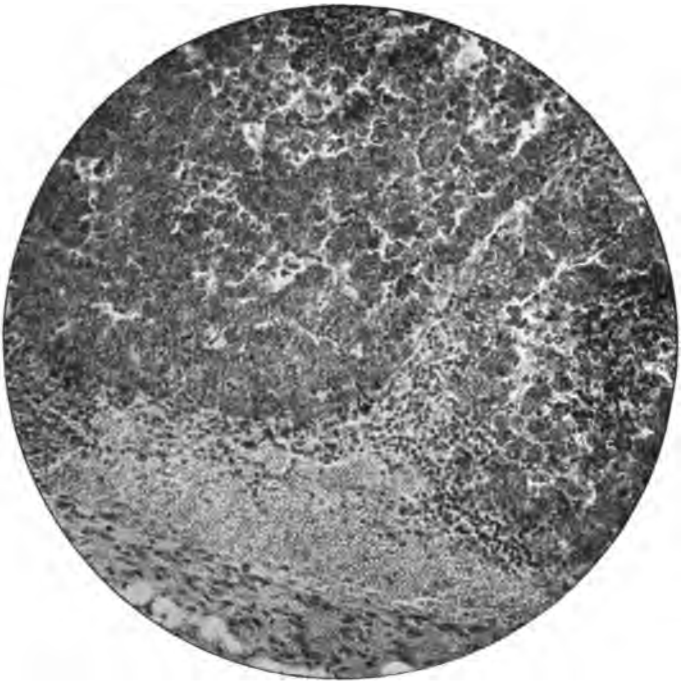

Fig. 1. Margin of retrograding tumor, showing hemorrhage. At margin of lobule at right, beginning atrophy and round-celled infiltration. Magnification 173 diameters.

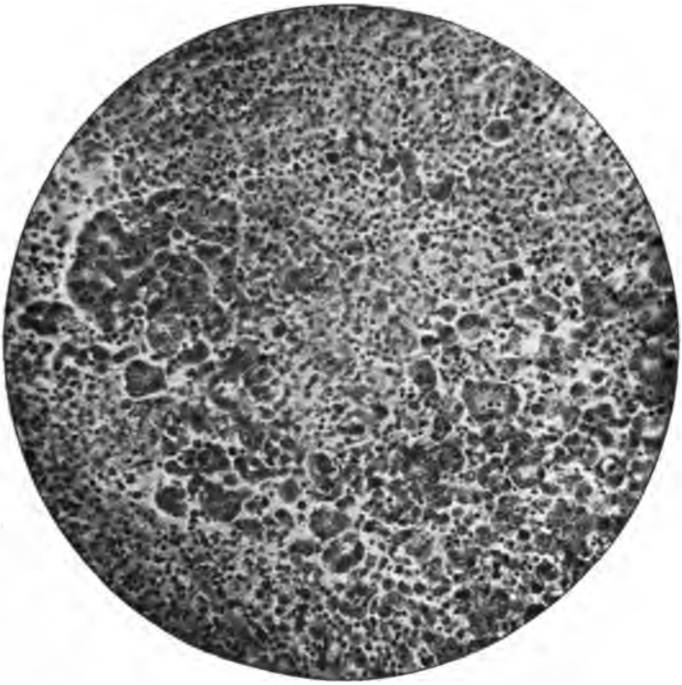

Fig. 2. Disintegrating alveolus taken from retrograding tumor shown in Fig. B. Cells broken up by connective tissue and hemorrhage. Isolated atrophic cells above and to the right. Magnification 173 diameters.

in isolated masses, between which are wide zones of connective tissue, the whole presenting the general outlines of an alveolar structure. Careful examination of margins of this necrotic material fail to show the presence of any epithelial cells. Growing between the necrotic remnants of epithelium are characteristic branching fibroblasts, and at the edge frequent connective-tissue phagocytes are seen, with remnants of necrotic material in their protoplasm. At the margin of some of the groups of necrotic cells are found large vesicular cells, the protoplasm of which is necrotic and stains palely, the nucleus of which can still be made out, and contains a certain number of chromatin granules. These are the only remnants of recognizable epithelium. Similar cells are occasionally found imbedded in the connective tissue. In some areas there are considerable aggregations of round cells lying between the large fibroblasts. Small hemorrhages into the connective tissue are occasionally seen. Some of the smaller, more condensed masses of necrotic material are surrounded by dense fibrous connective tissue, and at the margins of these areas characteristic foreign-body giant cells are to be found. This tumor obviously contains no growing epithelial elements. The distribution of the necrotic material in the connective tissue is such as to leave no doubt that it represents the remnants of a growing tumor.

The diagram of 213A (Plate II) has been included as illustrating the course of development of the majority of fatal tumors which usually cause the death of the mice in from six to ten weeks.

In accord with occasional observations in the literature of human cases, we have noted the occurrence of mouse tumors that first increase in size, and after reaching considerable dimensions, retrogress and apparently almost recover, then, after remaining stationary for a considerable period of time, once more increase in size, and after a more or less rapid development cause the death of the animal. 215B, 216D, and 340A illustrate this type of development.

215B (the diagram of which has been omitted on account of its great length) was inoculated March 28th, at the same time as other members of this series referred to above. Two small tumors first made their appearance on the back, in the region of the right hind leg, on the 10th of April. On the 17th of April five distinct tumors had made their appearance, which remained stationary for a while, and then diminished, two out of the five having disappeared on the 10th of May. On subsequent dates for several weeks, one tumor only was to be observed on the right side, diminishing gradually in size until a minimum was reached about August 4th. About this time, without any apparent cause, the tumor gradually increased in size until when killed on the 29th of September, the tumor was found to weigh 5.75 grams.

216D (see Plate II) is the most remarkable illustration of this type of development.

The tumor in this case was late in making its appearance, being first observed on the 5th of May, thirty-eight days after inoculation. It increased rapidly in size, a second tumor developed on the 10th of May, and on the 17th they were found to possess approximately diameters of 21 and 10 mm. Eight days later, the tumors were found to have retained about the same dimensions, having slightly changed their position, but on June 2d both had markedly decreased in size. On June 20th the lower tumor had entirely disappeared, and the larger tumor had fallen to dimensions of 3 x 2. From this point on a very gradual increase in size was to be observed, following a regular course, eventually causing the death of the animal on August 17th, on which date the tumor was found to weigh 7.6 grams.

340A (see Plate II) is another tumor which shows a remarkable period of retrogression between two periods of development, the latter of which terminated fatally.

The mouse was inoculated on the 26th day of May, 1905. Two small tumors appeared on the 14th of June, nineteen days after inoculation. On June 26th the maximum of the first stage of development was reached. There were four distinct tumors (see diagram), the lower tumor being the larger. Between the 26th and 30th of June all of these tumors retrogressed, the smaller one at the top showing the least amount of change. The lower or larger tumor had by this time so diminished in size that in all probability nothing but a connective-tissue structure remained, as is shown by its further fate.[1] On the 7th of July two of the smaller nodules had disappeared, the residue of the larger tumor remained almost unchanged in dimensions, but probably one of the upper two nodules had begun to grow, or the two may have coalesced, as is frequent, forming the new nodule, from which the second period of growth was derived. Eight days later the status was but slightly changed, and this state of affairs persisted until the 21st of July. Between the 21st and 31st, the residue of the larger retrograding tumor had diminished markedly, and the upper or growing tumor had increased somewhat. On the 5th of August the upper tumor had markedly increased; the lower tumor was stationary. On the 11th, the residue of the retrograding tumor had disappeared, the growing tumor had increased in bulk, and continued to increase steadily until the 11th of October, when the mouse was killed, the tumors at that time having reached large dimensions, as shown by the diagram.

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[1] That this is probably the correct explanation is indicated by the results obtained by Apolant in the treatment of large mouse tumors with radium. These tumors decreased rapidly until a certain size was reached, usually about that of a pea, after which they were slowly absorbed, and were no longer affected by the radium, due, no doubt, as Apolant concludes, to the fact that they no longer contained epithelium.

This case presents the apparent paradox of one tumor retrograding when another tumor is growing in the same animal. As will be seen by the histological evidence, in the later stages of retrogression the greater proportion of the residue of the tumor is connective tissue, and the most logical explanation of this phenomenon is that from the 30th of June the retrograding tumor no longer contained epithelium. About this time the immune forces of the animal failed, and the small unchanged nodule began to grow. Just why the larger tumor should have been more vulnerable than the smaller tumors, we are unable to state, but great discrepancies in the resisting power of tumors have been noted in animals, and have been observed in those human cases in which metastases undergo retrogression while the primary tumors are not markedly changed. The retrogression of tumors followed by subsequent growth does not occur very frequently in the mice, only five such cases having been observed in the course of the last twelve months, since accurate charting was commenced. It should be noted that in all five of these cases in which the tumor, after reaching considerable dimensions, first diminished and subsequently once more increased, the tumor had never entirely disappeared, but remained practically quiescent for many weeks before once more starting to develop. (See diagrams, Plate II, 216D, and 340A). No such recurrences have ever been observed in cases of complete spontaneous recovery from a true tumor. As to what is the exact nature of the forces which enable a small aggregate of cells to resist the action of the normal immune forces which have already resulted in the destruction of the residual portion of the tumor, is scarcely to be determined at present. We have no reason to believe that any external conditions to which the animals were subjected could have exerted such an influence upon their health and general condition as to lower the immunity sufficiently to enable the cancer material once more to develop with fatal results. It must, however, be borne in mind that the age of a mouse is an important factor which cannot be ignored. A tumor covering from five to six months in its development may be said to extend from one period of life into another, in the case of a mouse, the average of whose life is seldom above two years. Also, we are unacquainted with the absolute nature of the immune forces inhibiting the development of tumors and leading to their retrogression, and as to how long they last, and in what age they may be said to exert the greatest influence. It should be noted that all three of these tumors commenced to develop the second time during the warm weather, when, owing to high temperatures, the mortality amongst the animals was increased, and it is conceivable that the vitality or immune forces were on that account considerably diminished. We would not, however, attempt to explain the phenomena in question, but would rather put the results on record, in the hope that they may be of value in connection with future experiments along these lines.

HUMAN CASES.

Once aware of the frequency of spontaneous recovery from cancer in mice, one naturally turns to the literature, in search of spontaneous recovery in human beings. There are many cases of this sort in the literature, but very few of those recorded will withstand critical investigation. Many of them are obviously inflammatory lesions, syphilis, or Hodgkin's disease, which has been confused with sarcoma. There are others, however, which are, no doubt, true cases of retrogression of carcinoma, but these are unaccompanied by histological diagnoses, which fact renders them useless for critical purposes. Owing to the failure to distinguish between tubercular lesions and cancer, several cases reported in the early literature, which are accompanied by histological diagnosis, must be rejected on that account. The result of our search in this connection has led to the collection of fourteen cases, which, in our opinion, are authentic cases of spontaneous recovery. They include two epitheliomata, one scirrhous cancer of the breast, one malignant adenoma of the rectum, seven cases of chorion carcinoma, one endothelioma, and two sarcomata.

Zur Frage der Spontanheilbarkeit, des Krebses bei Menschen mit Demonstration. Senger. *Verhandl. d. deut. Ges. f. Chir.*, 1894, i, p. 171.

Senger first observed that an epithelioma of the tongue, which was directly opposite a sharp, decaying tooth, disappeared after the extraction of the tooth. With his attention thus attracted to the occurrence, some time later, when a patient, 42 years of age, with a tumor the size of a cherry on the mucous aspect of the cheek, opposite a decaying molar, presented himself, he determined to extirpate half of the tumor, and after removing the tooth,

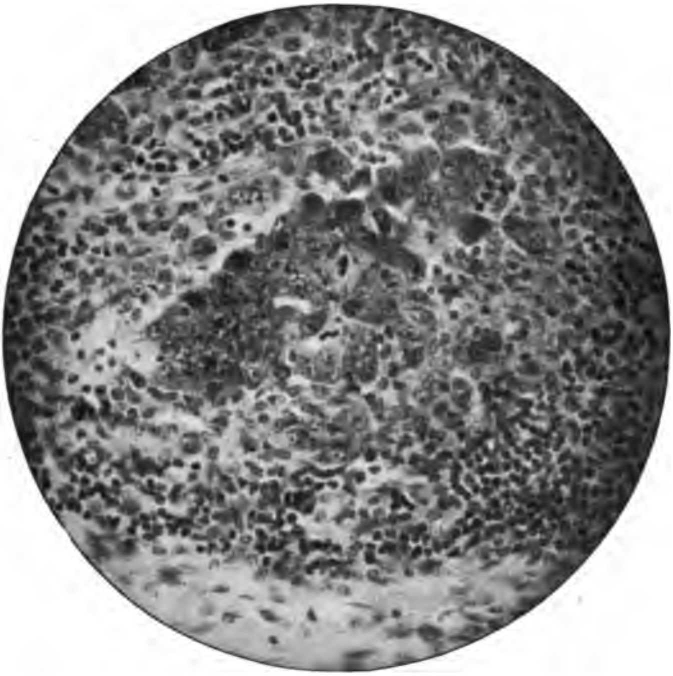

Fig. 3. Remains of a retrograding alveolus from retrograding tumor, with two karyokinetic figures. Magnification 260 diameters.

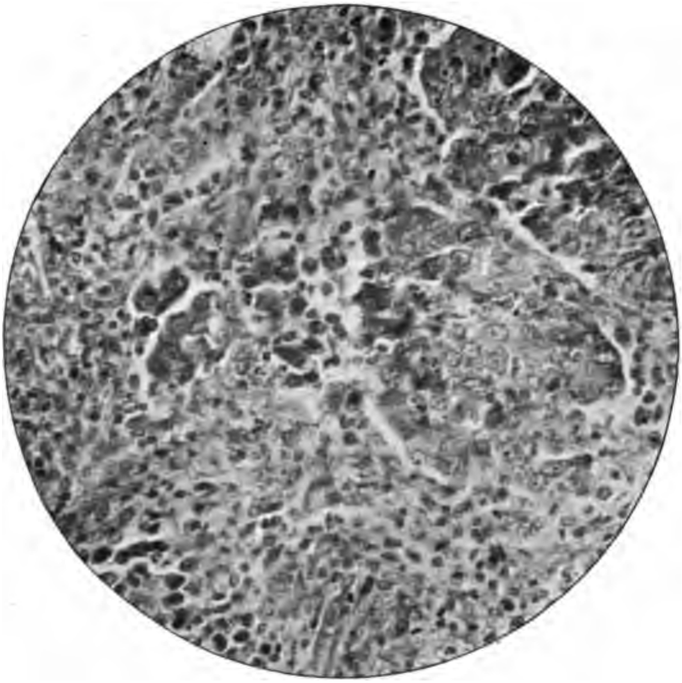

Fig. 4. Margin of a tumor retrograding under the influence of the X-ray. Atrophying epithelium, connective tissue growth, and hemorrhage. Magnification 260 diameters.

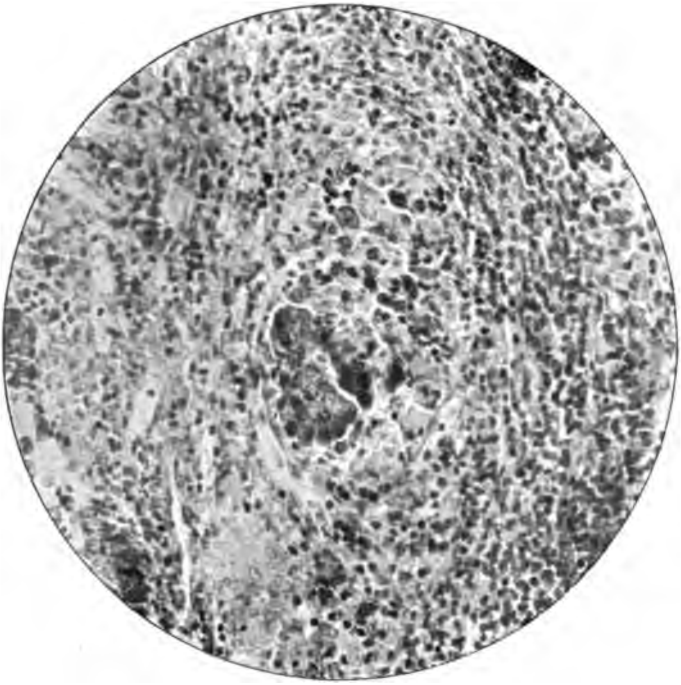

Fig. 5. Pseudo giant cell formed by coalescence of adjacent epithelial cells from retrograding tumor. High dry lens.

to see if the remaining portion would disappear. The center of the tumor, where the tooth pressed upon it, was slightly ulcerated. Its margins were elevated, hard on palpation and infiltrated the margin of the mucosa. The associated left submaxillary glands were enlarged and painful. Patient stated that the tumor had gradually increased in size during the preceding weeks. Half of the tumor and the adjacent muscle into which the tumor had infiltrated were removed at the time the tooth was extracted. The result was as he had anticipated. The remaining half of the tumor steadily decreased in size, and in three or four weeks nothing remained but a scar in the mucosa, and one year later, at the time of publication, there had been no recurrence. Illustrations of the sections of the tumor which accompany Senger's article show a typical epithelioma, with long papillary projections extending into the muscle, and nests of epithelium in the deeper layers of the submucosa. From the illustrations and descriptions there is no doubt that the tumor was a beginning epithelioma.

A case similar to this is the following:

A sore diagnosed as cancer of the lip in early life. Recovery without operation. By R. P. Crosbie, M. A., M. B. *British Medical Journal*, February 11, 1899, p. 338.

Patient, J. N., between 17 and 18 years of age, consulted Dr. Crosbie in May, 1898. His diagnosis was cancer of the lip. He had a diffuse eczematous affection of the lower lip, with a patch of ulceration about the size of a sixpenny-piece toward the right side. The ulcerated surface rested on a raised base, presenting numerous warty nodules. Patient stated that the sore had existed for six months, and that he did not smoke. There was no gland infiltration. The eczematous dermatitis of the lip was probably due to the irritating discharge, or from saliva, bubbles of which always appeared drying at the angles of the mouth. Patient was treated with antiparasitic ointments. Later, these were discontinued, and the condition of the lip grew rapidly worse. An examination was made of the tumor by the pathologist, Dr. Moore, who pronounced it malignant. Operation was advised, but Dr. Crosbie snipped off a prominent part of the ulcer with scissors, purposely leaving most of the growth, in the belief that it would heal. His expectations were justified, as on January 12th the eczema was cured, the infiltration disappeared, and all that remained at that time was a fibrous nodule, which Dr. Crosbie considered to be cicatricial.

A case of spontaneous disappearance of secondary cancerous growths. By A. Pierce Gould. *Clinical Society's Transactions*, vol. xxx.

M. C., a single woman, a sick nurse, until recently an inmate of the special cancer wards of Middlesex Hospital. In 1885, struck left breast with an umbrella; in 1888 noticed small lump in breast, which slowly enlarged, and in 1890 the mass was removed, and proved, on microscopical examination by competent observers, to be typical scirrhous cancer. In July, 1892, the left axillary glands became involved, and were removed. Later, in 1894, she was admitted to the Temperance Hospital, and was found to have several recurrent nodules around the scar, and considerable dyspnœa. She was considered inoperable, and was sent to the Middlesex Hospital. Her history, in January, 1895, stated that she was then 43 years of age, and for the past year had been steadily failing in health, and had lost considerably in weight. Examined by Mr. Lawson, who found on the left side of her breast numerous nodules involving the skin and subcutaneous tissue, and in the left axilla several hard enlarged glands; above the right breast a linear scar two inches long, in the center of which was a large nodule and in the right axilla a mass of hard glands. Enlarged glands were found above each clavicle. Patient complained greatly of dyspnœa of paroxysmal character; dullness over the base of the right lung, and numerous sonorous and groaning rhonchi on both sides. She continued for some time in a grave condition, with great dyspnœa and abundant expectoration. In December, 1895, somewhat better; winter of 1895–96, again in grave condition. When first seen in 1896 by Dr. Gould, she was unable to recline in bed, cyanosed, suffering from marked dyspnœa, cough, and expectoration, and sputum had contained blood. Many hard nodules in the skin of the left side grouped around the scar, and one larger in the scar above the right breast. Masses of enlarged glands felt in each axilla and above each clavicle. She complained of pain in the left thigh, and on examination it was found that this limb was an inch shorter than its fellow. the great trochanter raised above Neleton's line, and just below the trochanter the bone was greatly enlarged. In addition to the external recurrent cancerous growths, Dr. Gould believed that she had cancerous growths in the right lung and in the left femur, and he gave an absolutely unfavorable prognosis. On June 15th Dr. Gould examined patient again, and found one tiny nodule in the skin above the left scar. There were no enlarged glands to be felt in either axilla or above either clavicle. Her general condition was markedly improved. The dyspnœa disappeared; she could recline in bed. The dullness in the right chest had disappeared, except at the extreme base behind. The left leg was still shortened, but was less painful. From this period she steadily progressed, gained flesh, developed a good color, and enjoyed life. She now walks with a limp, but can rest her weight on the left leg. The limb is one and a half inches shorter than the right, there is an acute curve forward in the shaft of the femur, just below the trochanter, but there is no enlargement of the bone demonstrable. The right scar is still rather keloid, and its center is thicker than elsewhere. All skin nodules have disappeared, and there are no enlarged glands in either axilla or the neck. She received no special treatment. Gould believes that it is impossible to account for the change in the femur, except on the basis of a metastatic growth below the trochanter, which ultimately retrograded, the continuity of the bone being restored by the usual method of repair.

Polyposis recti-Adenoma malignum-Spontanheilung. Prof. J. Rotter. *Arch. f. klin. Chir.*, vol. lviii, p. 357, 1899.

Rotter describes a case of malignant adenoma of the rectum, which developed in a case of general polyposis of the sigmoid flexure and rectum, followed by spontaneous healing. Patient, Mrs. R., aged 31, entered the hospital September 9, 1895. Following last birth in January, patient had been troubled with chronic diarrhœa, which, in the last six weeks, had been associated with hemorrhage. Examination of the patient showed the presence of a fistula in ano, and about the margin of the anus mucous polyps from a hazelnut to a walnut in dimension. Examination with the finger showed that the entire circumference of the rectum was markedly transformed. The entire lumen was composed of rough, irregular elevations, from the size of a

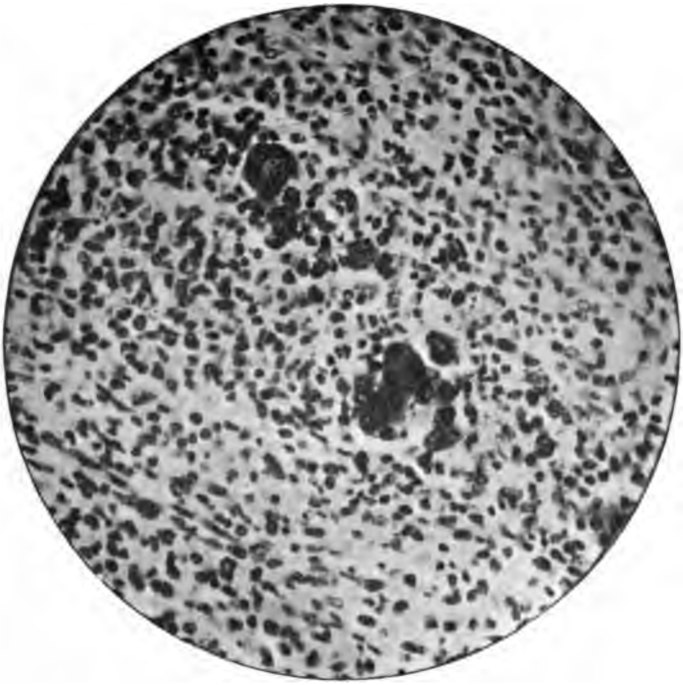

Fig. 6. Last remnants of epithelium imbedded in a large nodule of connective tissue in tumor 161L. Magnification 260 diameters.

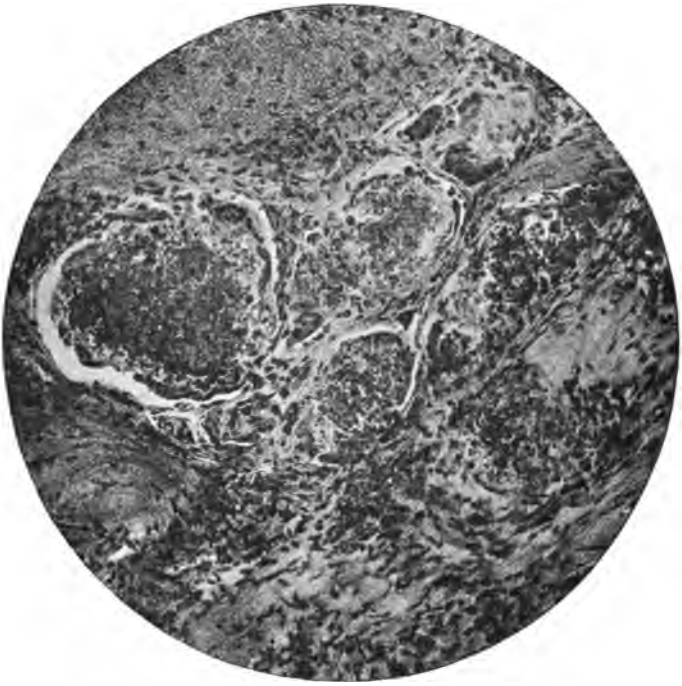

Fig. 7. Areas of necrotic material, with foreign-body giant cells and fibrous connective tissue found in the margin of a retrograding tumor which contained areas of extensive necrosis. Magnification 173 diameters.

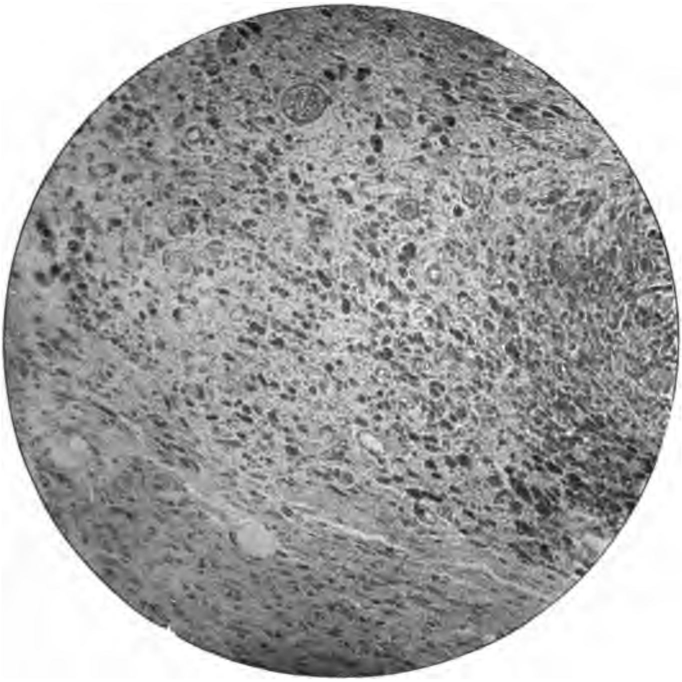

Fig. 8. Connective-tissue cells containing haematogenous pigment from the connective tissue surrounding a retrograding tumor. (Evidences of previous hemorrhage.) Magnification 173 diameters.

pea to that of a walnut, and at the lower margin numerous polyps, some of them with pendulous attachments which prolapsed through the anus. Between these masses the finger sunk into deep cleft-like depressions. The new growth involving the rectum, as well as the mucous polyps, had a comparatively soft consistence. Portions of the mass were easily detached with the finger, and the examination was associated with mucopurulent hemorrhage. Vaginal examination revealed the rectum as a more or less fixed, definitely palpable tube, the upper limits of which could not be determined. A microscopical examination (Orth) of the portions removed with the finger showed that the fragments were composed of extensively proliferated gland lumina, with here and there a cyst formation. The extensively proliferated epithelium, as well as the connective-tissue structure, showed a marked degree of mucoid degeneration. As the glandular structure was everywhere well preserved, the diagnosis of adenoma malignum was made.

On October 8, 1895, the fistula in ani was laid open with a thermocautery, and on this occasion several polypi, which were removed, proved, on examination, to be adenomata. Condition of the patient — diarrhœa, tenesmus, loss of sleep — strongly indicated the necessity of a radical operation.

For the purpose of determining how extensive was the involvement of the upper border of the rectum, an exploratory laparotomy was performed on the 10th. The exploration showed that the involvement of the rectum extended into the upper third, that both the sigmoid flexure and the colon were slightly thickened, but presented no evidence of tumor. Several polypi the size of hazelnuts, which were detected through the intestinal wall of the sigmoid flexure, were removed through small incisions into the gut. Through these openings it could be seen that the mucosa of the flexure was not as smooth as normal, but was covered with minute elevations. Having determined that the tumor was operable, on October 31, 1895, an extirpation of the rectum was performed. The tumor was found to be firmly adherent, the surrounding tissues markedly infiltrated, because of which, especially at one point, where it involved the posterior vaginal wall, a perforation was unavoidable. Ten to fifteen centimeters of the rectum, including the sphincter, were removed, the gut from above being drawn down and united to the skin. Recovery was without incident. During the end of December a slimy granulation area was developed in the lower portion of the wound, which, during the middle of January, filled in the entire space between the rectum and vagina, and protruded through the perforation into the vagina to such an extent that it developed a mass within the vagina the size of a hen's egg. No new excrescences could be detected above this within the rectum. Because of the inconvenience of this enormous mass, on January 5, 1896, the greater portion was removed with a sharp spoon. The microscopical examination (Orth) of the recurrent tumor showed the same histological characteristics as those of the primary tumor. The growth promptly returned, and both in February and April, had to be removed by curettage. Promptly following the last curettage, the growth returned so rapidly that she was discharged from the hospital as incurable.

Examination of the extirpated rectum, the lower margin of which was two centimeters from the lacunæ Morgagni, and the upper end fifteen centimeters above. The tumor consisted of masses from the size of peas to that of walnuts, with broad bases, the surface very irregular, and many areas of ulceration and necrosis; between the elevations, deep depressions and clefts, in which no evidence of malignancy could be detected. In the lower portion of the preparation, numerous polyps, the whole presenting the characteristic picture of a soft mucoid carcinoma of the rectum. A section through the rectal wall showed that the muscularis was no longer recognizable; that the epithelial proliferation was surrounded by a mass of scar tissue.

After the discharge of the patient in April, 1896, in July, 1896, she returned to the clinic for examination, and to Rotter's astonishment he found that the large cauliflower growth about the anus had totally disappeared, nothing being left but a perfectly smooth scar. On questioning the patient, it was found that the tumor had gradually decreased in size and had ultimately disappeared.

In considering this case, Rotter points out that the tumor presented all the clinical characteristics of malignancy; the destruction of the muscular coat of the intestine, the infiltration of the surrounding tissues including the wall of the vagina, and the repeated recurrence at the site of the original tumor leaving no doubt in his mind of its malignancy.

Three years later (*Arch. f. klin. Chir.*, vol. lxi, p. 881) the patient died of a metastasis on the surface of the right iliac bone. On microscopical examination of the metastasis, it presented the characteristic structure of the mucoid adenoma malignum which developed in the rectum and which had recurred and finally spontaneously disappeared. An examination of the colon, including the region of the operation, showed a definite, smooth scar at the point where the recurrence had taken place and no evidence of return. The entire colon presented the characteristic appearance of advanced polyposis. A miscroscopical examination (Orth) of a number of these polyps showed them to be of a benign character. Rotter was of the opinion that the tumor mass on the surface of the right iliac bone represented a belated recurrence of the rectal tumor. Although he repeatedly points out in his article that benign polyps, such as those which lined the entire colon, frequently underwent malignant degeneration, he was more inclined to believe that the recurrence was from the original tumor than from a possible later degeneration of these benign structures. Patient appears to have been free from recurrence for a greater portion of the three years. He considered the ultimate recurrence of the disease as proof positive of its malignant nature.

Case of spontaneous arrest of growth in an endothelioma, with subsequent inflammatory absorption. B. M. Randolph, M. D. *Proceedings of Pathological Society of Philadelphia*, vol. vii, No. 4.

M. S., aged 43. Three years before operation, patient developed on the posterior aspect of the left forearm, at the junction of the upper and middle thirds, a nodule beneath the skin, which was movable and painless. The mass grew steadily and painlessly for six months, ultimately becoming a flattened slightly elliptical mass 1½ x 2 x ¾ inches in size. For two and a half years, approximately until two weeks before the operation, it remained stationary. At this time it became painful, and there were signs of inflammatory reaction about the tumor. The tumor was excised. Wound healed by primary union.

Pathological Report.— Tumor is beneath the skin, which is not adherent to it. It is not encapsulated. It has an envelope which is derived from the surrounding connective tissue. It presents a grayish white appearance.

Microscopical examination revealed the presence of marked areas of necrosis. Among these are other areas, in which the necrotic process has not taken place. Here the alveoli are filled with medium-sized round cells with round nuclei staining with moderate intensity. No connective-tissue fibers were demonstrated between the cells, and Randolph concluded that the growth was evidently derived from the endothelium of the lymph spaces. The connective-tissue stroma revealed a remarkable phenomenon. There was active hyperplasia with very marked round-celled infiltration. New-formed blood-vessels were numerous. About the margins of the necrotic cell areas were numerous giant cells, sometimes completely sūrrounding the alveolar spaces. Randolph believed that they were phagocytic.

Two cases of sarcoma, with histological examinations; one from the older literature, 1876, which arouses some doubt as to the diagnosis, the other being perfectly satisfactory. R. Tripier. *Lyon Medical,* 1876.

A. S., 22 years old, gardener. Entered the Hôtel Dieu, June 11, 1875. Examination by M. Tripier, June 24th, showed in the epitrochlear and epicondylian regions of both limbs the presence of a number of small nodules in the neighborhood of the superficial muscular tissue. In the biceps, in the superficial muscular tissue, and in the interspaces, and principally along the sheath of the vessels, a large number of small spherical or ovoid tumors from the size of a pea to that of a hazelnut. The tumors are fairly mobile, slightly elastic, and fairly solid and resistant. Pressure upon them causes no pain. The pectoral muscles of both sides contain a large number of tumors similar to those in the arms. Most of them are deeply seated, but one in the anterior and middle part of the right arm appears to be subcutaneous. The lower limbs are involved to a lesser degree. The disease, according to the patient, appears to have begun in 1874, and led to such pains and muscular stiffness at the time of the onset as to oblige the patient for a time to cease work. The patient was placed upon potassium iodide, but the stomach being unable to tolerate it, its use was suspended. July 27th, patient complained of pain in the side, and had a slight pulmonary hemorrhage. Pulmonary hemorrhages from this time on were frequent, and the tumors in the lower limbs increased in size and number. The treatment of iodide and Fowler's solution was discontinued at this time and cod-liver oil substituted, with some improvement in the general state. Strength and appetite returned. Microscopical examination of one of the specimens, which was extirpated, was made, and the case was considered hopeless. In February, 1876, the tumors had greatly diminished in size, and by April, 1876, only one small tumor the size of a pea could be detected on the internal side of the left arm. The movements were now free. Patient was seen in 1881 by M. Tripier. At this time he was in good health, no tumor having reappeared. M. Morat made the histological examination of the tumor removed from the body of the left biceps. When stained with picrocarmine the section proved to consist of longitudinal bundles of fusiform cells containing one or more nuclei. Many of the cells have extensive prolongations. Blood-vessels are fairly numerous. Tumor was diagnosed by Morat as certainly a sarcoma.

A case of recurrent sarcoma, with apparently spontaneous cure and gradual shrinking of the tumor. By A. Laurie Watson, M.A., M.B., Ch.B., Glasgow. *The Lancet,* 1902, i, p. 300.

The patient, a strong, healthy-looking woman, aged 36 years, admitted to Glasgow Royal Infirmary, November 21, 1900. She was absolutely free from ailment, and came into the hospital because a swelling on the back was annoying her, and she wished to have it removed. The tumor was large and pendulous, being attached to the left side of the back by a base nine inches wide, and rather less than four inches in vertical measurement. The apex hung down for almost four inches below the lower margin of its attachment to the back, while the upper margin was just below the inferior scapular angle. It was soft, but not fluctuant, slightly nodulated, and had the appearance of a lipoma undergoing degeneration; the skin covering the dependent portion was of a dark reddish color. The tumor was regarded as simple, partly because of its character and partly because its history went back to the patient's childhood, though it had grown considerably in recent years.

On November 23d, Dr. D. McK. Dewar, acting for Dr. D. Newman, removed it freely with all the discolored skin, and remarked at the time that in three respects it differed from an ordinary lipoma: 1. The tissue was of a dirty grayish-yellow color, and very friable; 2. It was exceedingly vascular; and 3. Its margins were diffuse and indefinite. After the operation, hemorrhage was so free that the dressings had to be frequently changed in the first few hours; thereafter they were left for four days untouched. On the 27th it was observed that the dressings had become tight, and on exposing the back the tumor was found to be almost as large as before the operation. In the expectation that this might be due to accumulation of blood-clot beneath the flaps, a portion of the wound was opened, but no blood could be expressed, even when a director was introduced and worked about inside the wound. It was therefore concluded that the swelling was due to a rapid re-formation of the tumor. By an unfortunate oversight, the tumor first removed had been destroyed without microscopical examination; but at this stage two small portions of the tissue were removed. One of these I hardened and stained myself; the other was sent to Dr. C. Workman, pathologist to the infirmary. The sections obtained from the first portion were examined by Dr. Newman, by Dr. Dewar, and by myself; and the recognition of sarcomatous tissue, chiefly round-celled, was unhesitating. Dr. Workman's quite independent report described the tissue as a mixed round-cell and spindle-cell sarcoma.

A week later the operation wound had begun to ulcerate along its margins, and it was decided to attempt a wider removal, but when, on December 4th, the patient was placed on the table and anæsthetized, the tumor had attained such a size and was so diffuse in its margins that further radical interference was considered out of the question; the ulcerated edges were therefore cut away and the wound was again stitched up. During the remaining days of her residence in the infirmary the patient looked and felt very weak and ill. A bad prognosis was given, and she was dismissed on the 13th. After this several medical men saw her, and all took a gloomy view of the case; but in spite of everything she improved, and a month after dismissal from the hospital a letter was received from her own medical attendant stating that the operation wound had almost completely healed. When I visited her at her home in the country on February 9, 1901, I reported as follows: "The operation wound has entirely healed up, with only a small scab at its lower extremity, and leaves a firm, healthy-looking cicatrix. The tumor is much less tense than at the time of dismissal, and shows a tendency to return to the pendulous form which it had when first seen; it is flaccid and quite painless. At its anterior margin are some subcutaneous livid patches, which were noticed at the time of the recurrence of the tumor, but these do not appear to have changed at all or to be spreading. The patient's general condition is much improved; she is able to be up and about, eats with a good appetite, and in general feels very comfortable." She again reported herself at the infirmary in April, and the tumor seemed to be still further diminished in size.

Finally, I again visited the patient at her home on November 21st, exactly a year after her admission to the infirmary, and the following is my report: "The general health is excellent. The tumor is much less prominent, and hangs like a loose sac, as if almost all its contents had been evacuated. There is slight lividity at the margins, but no pain, except an occasional stinging, like neuralgia in damp weather. The tumor covers about the same area as at the time of dismissal, but consists of little more than loose skin. The scar of the operation wound is perfectly sound and firm. No pain is caused by free manipulation, and the clothes are worn without discomfort."

It is interesting to find that one of the most malignant forms of cancer — chorion carcinoma — should furnish us the greatest number of authentic examples of spontaneous recovery. This is particularly interesting because this type of carcinoma is most frequently subjected to microscopical examination. The frequent occurrence of spontaneous retrogression has introduced some confusion in the literature of chorion carcinoma. Cases which have been positively diagnosed as malignant have undergone retrogression, and we find that an active discussion is in progress as to what constitutes the evidence of malignancy in this tumor. In the light of the observations made on the mice, it would appear that there is no reason for assuming any essential difference between those tumors which retrograde and those which terminate fatally, aside from the resistance of the individual. Most of these cases have been subjected either to a simple curettage or an incomplete operation followed by recurrence.

The first illustration of retrogression of chorion carcinoma is found in Marchand's second article,[1] which, with his first,[2] constitutes the basis of our present classification of this form of tumor. In this case, the chorion epithelioma had penetrated through the uterine wall, and had so firmly infiltrated the pelvic tissues behind, that it could be but incompletely removed.

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[1] Monatschr. f. Geb. u. Gyn., bd. i, h. 5 and 6, 1895.

[2] Zeitschr. f. Geb. u. Gyn., bd. xxxix, h. 2, 1898.

Furthermore, a thrombosis of the uterine vein in the right lateral ligament which was severed during the operation was found to contain masses of proliferating epithelium, so that Marchand expressed the opinion that recurrence and metastases would shortly follow. At the end of one year, however, neither the abdominal organs nor the lungs contained metastases, and the patient presented all the evidence of complete health. Undoubtedly, according to Hörmann, in commenting on the case, the masses of tumor which were not removed were deprived of their malignancy, so that they were no longer harmful to the patient.

Hörmann gives complete details of a case, in an article in Hegar's *Beiträge z. Geb. u. Gyn.*, bd. viii, h. 3, 1904, "Zur Frage der Bösartigkeit und über Spontanheilung von Chorioepitheliomen."

H. E., age 36, IV para, was admitted, 1901, into the University Gynecological Clinic, Munich.

Patient's periods have been irregular since the 15th of July, 1900. After a two months' pause, on the 15th of September she had an excessive hemorrhage from the uterus, and during eight days preceding the admission to the hospital, constant but not excessive hemorrhage. On the 18th of September, 1900, she had passed lumps of tissue and masses like portions of skin. She was treated for subinvolution of the uterus following abortion, with hot douches, etc. Hemorrhages ceased, six weeks afterward again returned, and on the 12th of November she had a very active hemorrhage. The uterus at that time was the size of an orange, its surface smooth. A curettage was performed at this time, and the microscopical diagnosis was as follows: Endometritis post-abortum, definite islands of decidua, and granular hypertrophy. After curettage the hemorrhage decreased for a considerable period, but ultimately returned in irregular form. Hemorrhage frequently contained dark brown fragments of tissue, sometimes masses of whitish substance. The uterus was again the size of an orange. At the time of her admission in March, 1901, she complained of constant hemorrhage, pain in the back, shortness of breath, and cough without expectoration.

The examination showed that on the anterior aspect of the vaginal wall, somewhat to the right, were two prominences the size of cherries, with necrotic surface and livid discolored margins. They presented the appearance of metastases. The uterus is larger than a man's fist, rests upon the anterior vaginal wall, and corresponds to a mass felt above the symphysis, which on palpation is found to have nodular outlines, and the whole is firmly fixed.

Because of this fixation, and the evidence that the uterus is involved, as well as the surrounding tissues, a radical operation is considered to be impossible, therefore the two vaginal nodules were removed with a cutting Paquelin and a curettage of the uterine mucosa performed. During this procedure the uterine cavity was found to be greatly enlarged, the surface raw, and during dilatation bloody brown masses of tissue, as well as proliferative tissue resembling mucosa, were extruded from the uterus, and greater masses removed with the curette.

The two metastases in the vaginal wall were immediately hardened for examination. Under the microscope both the vaginal metastases were found to consist of typical chorion giant cells and residual masses which penetrated deep into the muscular structure and produced atrophy and destruction of the muscular fibers. The diagnosis of chorion carcinoma of the uterine body with metastases in the vaginal wall was made, and because of the nature of the tumor an absolutely unfavorable prognosis was given. During the clinical observations which followed the operation, there were several times symptoms which pointed to the formation of pulmonary metastases, pain in the lungs, constant coughing without expectoration, cramps, dyspnœa, and marked cyanosis of the face. On the sixteenth day the patient requested to be permitted to return home, where she was later visited after some weeks by Hörmann. She was reported at one time to have had blood in her sputum, she became rapidly cachectic, and was confined to her bed. On the 29th day of April, 1901, she was taken into the Krankenhaus r. d. Isar. Here a metastasis the size of a cherry was removed from the introitus (entrance, dicht über der Harnröhre). Obviously, this was a recurrence of one of the earlier nodules. The uterus was very large, reached to a point half-way between the symphysis and the umbilicus. The patient constantly passed hemorrhagic mucopurulent secretion, had repeated chills, and a temperature of 41° C. On the fourth day of May, a new curettement and removal of the vaginal nodule.

Diagnosis of the tissues from the uterine cavity and from the vagina was made by Professor E. Albrecht as undoubted chorion carcinoma. From this time on Hörmann lost sight of his patient, but recent publications on the spontaneous cure of chorion epithelioma reawakened his interest, and he searched for the patient, and was not astonished to find that more than two years after the operation she was, at the time of his publication, in absolute health, with rosy cheeks, and with a strong young baby in her arms, which had been born a few weeks before, in a maternity institution in Munich.

On carefully looking over the records of her case, he found that, following the second curettement, the only treatments she received were constant uterine irrigation, that the discharge from the uterus gradually decreased, appetite and general condition gradually improved, and at the end of eight weeks she was able to leave her bed. A run of temperature which had continued, reaching as high as 38° and 39°, gradually subsided, the uterus gradually decreased in size, the cervix gradually contracted. At the end of three months the patient was in fairly good health, the uterus was reduced to the size of a pear, was mobile retroflexed, and the parametrium and adnexa were free from disease. The hemorrhagic discharge had long since ceased, and no metastases were evident.

Beginning with August, 1901, to August 11, 1902, her periods returned regularly at the usual period, and lasted two days. At the end of this period she became gravid, and the course of pregnancy was normal in every way. On the 22d of May, 1903, she gave spontaneous birth to a mature fœtus, weight 3,750 grams. The placenta and membranes were absolutely without pathological change. The puerperium was without incident; the periods returned six weeks after the birth.

Examination held on July 21, 1903, by Hörmann, is as follows: Extremely good appearance, exceptionally good nutrition. Over the entire pulmonary region, pure vesicular breathing; heart tones good. Abdomen, soft abdominal wall; absolutely no resistance over the symphysis. Vaginal examination shows the vaginal wall absolutely smooth; nowhere evidence of tumor or remains of such; no evidence of scars. Portio in the spinal line, cervix a closed transverse cleft. Small amount of mucofluor cervicalis. Uterus undergone complete involution, slightly larger than a hen's egg, of good consistence, lying upon the anterior vaginal wall. In the anterior vaginal wall three or four small myomata the size of peas. The adnexa are free from inflammatory changes; the uterus absolutely movable.

Two cases of deciduoma malignum. Noble. *American Journal of Obstetrics*, vol. xlvi, No. 3, p. 289.

Noble reports, under the heading of case 2, an undoubted case of spontaneous retrogression of chorion carcinoma. Mrs. D., aged 24, two pregnancies. The first ended in miscarriage November, 1899, the second at the sixth week, August 15, 1900. During the past-year menstruation has been irregular. Since the last miscarriage, August 15, 1900, constant hemorrhage from the uterus, profuse during the first two weeks, variable since. Entered Kensington Hospital for Women, November 14, 1900. Vagina and cervix normal. Large tumor resembling fibroid attached to the cervix and filling pelvis. Hysterectomy was performed November 17th. On opening the abdomen it was evident that the tumor was malignant and attached to the fundus. It filled the pelvis, was of dark bluish mottled appearance. It was adherent to the omentum, and appeared to have infiltrated the fundus of the bladder. Diagnosis of deciduoma malignum was made from the history and the appearance of the tumor. On attempting to separate the tumor mass from the bladder, the finger of the operator entered the soft structure of the tumor, and it was necessary to leave a mass of the neoplasm attached to the fundus of the bladder. On account of patient's feeble condition the operation which would have been necessary to remove completely the growth, including a large portion of the bladder-wall, seemed contraindicated, and a supravaginal hysterectomy was performed with the view that the case was hopeless from the standpoint of radical cure. Gauze drain was introduced. Convalescence was febrile until the sixth day, after which it was uninterrupted. February 12, 1901, three months after the operation, patient had regained the appearance of health, and expressed herself as feeling well, and on pelvic examination, vagina was found to be normal. A distinct mass might be palpated upon the bladder-wall where the portion of the tumor had been left after the operation. March 26, 1902, sixteen months after the operation, patient was again seen. She presented every evidence of good health, and expressed herself as quite well. Careful examination failed to show any evidence of malignant disease in any portion of the body. The mass which had been felt on the bladder at the preceding examination had disappeared. Vagina, bladder, and broad ligaments were entirely normal to touch.

The tumor removed at the operation was submitted to a careful microscopical examination. Sections taken from the periphery of the growth were described by the pathologist, Dr. Babcock, as follows: At the periphery, irregular masses of large cells are found infiltrating the muscular wall of the uterus, apparently lying in distended uterine sinuses. These cells contain one or several oval, rounded, or irregular, or more frequently fusiform, nuclei. In certain of the cells karyokinetic figures are present. The nuclei are various sizes. The surrounding protoplasm is finely granular, large in amount, and there is no distinct cell-wall, so that when massed together the demarkation between adjoining cells is not perceptible. Adjoining these cells, and frequently infiltrating between the muscular fibers, are much smaller cells, with small, regular, rounded nuclei encircled by a narrow band of protoplasm. These are not universally present, and are not nearly as conspicuous as the larger cells. The uninvaded portions of the uterine muscle show sinuses and blood-vessels distended by blood. Invasion by small lymphoid cells seems to precede that of the larger cells. As one recedes from the periphery, masses of the larger cells are found imbedded in the fibrine and amid collections of red corpuscles. There is only a moderate infiltration of polymorphonuclear leucocytes.

Section from the mucous membrane of the cervix uteri. The epithelial lining of the cervical mucosa is absent, and apparently had been removed by the curette. There is a slight hyperplasia of the uterine glands, and in some of these a tendency to a desquamative catarrhal process is indicated by the loosened cells and the granular *débris* found filling the cavity. About a few of the glands there is an infiltration of small cells of the lymphoid type.

The pathological diagnosis is syncytioma malignum of the corpus uteri.

The above description, combined with the two excellent illustrations given by Noble, leaves absolutely no doubt that his case was one of true chorion carcinoma.

Littauer. *Arch f. Gyn.*, vol. lxxii, p. 294.

Littauer reports a case in which six weeks postabortum, an examination of the cavity of the uterus, from which there had been constant hemorrhage, discovered on the posterior wall of the uterus a circumscribed, soft nodule the size of a cherry, which pentrated through the uterine wall directly beneath the serosa.

Microscopical examination of this tissue, which was made by Risel, showed that it consisted of a proliferation of the epithelium of hydropsical chorionic villi. The cells which presented the characteristics of the giant cells of the serotina had penetrated between the muscle fibers, in many cases causing atrophy of the latter. In many of the smaller blood-vessels the masses of epithelium could be found penetrating the vessel-walls, which undoubtedly accounted for the excessive hemorrhage. A removal of the uterus was recommended, but as the condition of the patient improved rapidly, this was not performed. Several weeks later, a second curettage was made, with the result that the uterine mucosa was found to be perfectly normal, and one year after the first curettage the patient was perfectly well.

Fleischmann. *Monatsschrift f. Geb. u. Gyn.*, bd. xvii, h. 4.

Two and a quarter years after the removal of a hydatidiform mole, there developed in the wall of the vagina a bluish-red nodule the size of a chestnut. The enlarged uterus was found to contain friable tissue, and as a radical operation was refused, the nodule was excised and the uterus curetted. During the curettement a perforation of the uterus on the right side was followed by the development of a hæmatoma in the right lig. latum.

The microscopical examination of the nodule from the vaginal wall, as well as the material obtained by curettage, gave undoubted evidence of chorion carcinoma. Predominating type was of the syncytial variety. (See illustrations.) The majority of the material removed by curettement consisted of malignant tissue forming large complexes of cells penetrating between the uterine muscular wall, the cells of which were in many cases widely separated by the proliferation, and several preparations showed marked atrophy, cloudy swelling, and necrotic areas. Microscopical appearances were such that both Marchand and Fleischmann made an absolutely unfavorable prognosis. Three months later the patient presented every evidence of health. Ten months after the operation, absolutely no pathological changes could be found in the genital tract. Menstruation had been established four months, was normal and regular. Fleischmann was of the opinion that the criticism that the curettement might have removed all of the tumor mass must, on the basis of the histological findings, be negatived.

The patient has since borne two children.

Von Franqué. *Zeit. f. Geb. u. Gyn.*, bd. xlix, h. 1.

Von Franqué publishes a case in which three months post-partum the patient's uterus was considerably enlarged. Curettement removed large quantities of friable tissue, which, on microscopical examination, he concluded to be typical chorion carcinoma. Following the curettement, the patient's health was restored. She has twice since given birth to normal children, and at the end of six years is perfectly well.

Langhans. *Beitr.z Geb. u. Gyn.*, bd. v, h. 1, 1901.

Langhans reports a case in which, following a normal birth, a vaginal nodule the size of a walnut developed.

This was removed with a sharp spoon, the base being cauterized. Fourteen days after, a recurrence developed at the site of the original nodule. This recurrent nodule broke open, cast out a green, necrotic mass of tissue, and healed spontaneously. Microscopical investigation showed, besides necrotic tissue, well-defined syncytial masses, between which are cells obviously derived from the layer of Langhans. Ten months later the patient appeared to be well.

Besides the fourteen cases here collected of cure, histological evidence of retrograde processes affecting the margins of tumors have been reported by Becher, Petersen, and Schwartz. Becher (Über Riesenzellenbildung in Cancroiden, *Virchow's Archiv,* 156, p. 62) described changes at the margin of squamous-celled epithelioma of the skin, which, to his mind, indicated a spontaneous retrogression of the epithelial pearls, and other adjoining epithelial cells, and he also found about the margins of these atrophying and hornifying cells, numerous giant cells, both of the myeloplax and the Langhans type. He believed that these giant cells were of the foreign-body type, and attributed to them both amœboid and phagocytic activity, believing that they were removing the atrophying epithelium, as in the case of other foreign bodies. He held that through the phagocytic activity of these giant cells a process was inaugurated which, in many cases, led to a form of partial spontaneous cure, resulting in the total disappearance of the epithelial pearls, and the organization of the same with young connective tissue. He believed that certain etiological factors in the development of the tumors favored this process, especially the slow growth of squamous epithelioma and the chronic inflammatory irritation, associated with endarteritic changes in the blood-vessels, assisting in the isolation of the epithelial nests. Besides the foreign-body giant cells, Becher recognized a form of giant cells derived by coalescence of groups of isolated atrophying epithelial cells. He compared the changes found in these cells with those produced experimentally by L. Loeb,[1] who transplanted fœtal epithelium into the subcutaneous tissue of adult animals of the same species. The cells of these transplanted fragments maintained themselves for a certain period in the tissue, and gave evidence of having divided for several generations. Upon the exhaustion of their proliferative power, Loeb found that the cells showed marked signs of atrophy, the borders of adjacent cells becoming obliterated. The nuclei underwent fragmentation, and the whole coalesced to form a pseudo giant cell, which in its ultimate form was scarcely distinguishable from a typical giant cell of connective-tissue origin.

At the termination of his article he stated that although the process of partial spontaneous cure in cancer inaugurated by or associated with giant-cell formation, in the ordinary clinical sense, was without much significance, it was, however, not improbable that, under certain favorable conditions, this process might lead to a clinical cure. He viewed this process as an evidence of the natural resisting power of the body.

Following the suggestive observations of Becher, we are indebted to Petersen for further evidence on this subject.[2] Petersen laid special stress upon the atrophy and coalescence of groups of cancer-cells as evidence of more complicated processes, which, under given conditions, could rob the cancer-cells of their vitality, and viewed these changes as direct evidence of special retrograde processes of the epithelium. He accentuated Becher's references to Loeb's experiments, and pointed out that besides the ordinary processes of fatty degeneration and necrosis which often involved the central portions of çarcinomata, there was evidence at the margins of the epithelial cells that they were deprived of their vitality by some obscure process.

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[1] Arch. f. Entwickelungs Mechanik der Organismen, bd. xiii, h. 4.

[2] Beiträge zur Klin. Chirurgie, 1902.

Schwartz describes an epithelioma papillare (*Virchow's Archiv,* bd. clxxv, h. 3, 1904) which developed in the scalp, apparently from and in the neighborhood of atheroma cysts. This tumor was of considerable extent. It was removed by operation, was a cauliflower-like growth, the base measuring 6 x 4 cm. The wound healed promptly, and the patient was discharged cured. The tumor was of papillary epitheliomatous character, and showed extensive areas of hornification, and in other portions partial calcification. Through the entire tumor there were areas of marked organization, which appeared to have developed in a short period of time, and to have led to a healing of considerable portions of the tumor. He describes at length the evidence of spontaneous retrogression of certain alveoli of this tumor, finding practically the same conditions as Becher. He describes accurately the type of giant cells as noted by Becher, and later by Petersen, but calls attention to the fact that many of the alveoli were in course of organization by connective tissue without the presence of giant cells. In this connection he refers to cases of Franke and Denecke, in which this phenomenon was marked. Franke described a case of epithelioma associated with atheroma cysts very much like that of Schwartz. Large portions of this tumor were invaded by young connective tissue, presenting the appearance of the organization of the epithelial nests by granulation tissue. Denecke, in a case of calcified epithelioma, likewise described the organization of portions of the tumor by connective tissue. The presence of giant cells were noted, and these appeared to be about the calcifying masses of atrophied endothelium. Borst also calls attention to a tumor which in places presented evidences of partial organization.

It will be seen from these publications that reparative processes in carcinoma were first recognized by Becher, a student of Orth, whose opinions were substantially indorsed by Petersen, and that further evidence of spontaneous curative processes has been added by Schwartz, a student of Orth. Both Becher and Petersen state that, in their opinion, these processes, when sufficiently energetic, lead to a complete clinical cure of established cancerous processes.

A comparison of the histological findings in the retrograding mouse tumors with those observed by Becher, Petersen, and Schwartz, in human tumors, shows that the changes found at the margin of all retrograding tumors are the same. We find the same pseudo giant cells resulting from coalescence of the atrophying epithelium, the foreign-body giant cells at the margins of necrotic material where this is present, and the growth of connective tissue; from all of which we may assume that the agencies which are responsible for the retrogression of the mouse tumors are the same as those which are occasionally active at the margins of human carcinoma. It has been found that a mouse, once recovered from carcinoma, cannot be successfully reinoculated within a certain period, and from this it is clear that the animal is, under these conditions, endowed with a resistance or an immunity against cancer which it did not previously possess. That the inhibiting effect exerted by these immune forces on the development of tumors is not always successfully maintained is shown in the case of those tumors (see diagram, Plate II), which, for a period, remain stationary or retrogress, and then ultimately overcome the resistance which is opposed to them, and succeed in destroying the life of the animal. It would appear that evidences of retrogression at the margin of growing tumors are the expression of insufficient activity of these immune forces. Whatever the nature of this immunity, it does not appear to be cytolytic in its action, the histological picture indicating rather that the epithelial cells at the margins of the tumor are in some way robbed of their power of destructive proliferation, after which they undergo simple atrophy and gradual absorption by the surrounding connective tissue. There appears to be some relation between the areas of hemorrhage at the margins of retrograding tumors and the atrophy of the cells, the evidences of retrogression being usually most marked at these sites. From the fact that the treatment of cancer materials previous to inoculation with the blood of recovered mice considerably reduces the percentage of successful inoculations, it may be supposed that the extravasation of blood into the tissues liberates an immune body capable of acting upon the epithelium.

In this connection it is interesting to note that mouse tumors retrograding under the influence of the X-ray present the same histological picture as those retrograding spontaneously, although the blood of such mice does not appear to exert an immune action upon cancer material. How the X-ray succeeds in bringing about retrogression of a tumor has been the subject of much discussion. Some authors have held that it interfered with the processes of karyokinesis. The detection of karyokinetic figures in the last remnants of retrograding alveoli is opposed to this interpretation of its action. On the other hand, Perthes'[1] experiments with warts appear to give us a more definite clue to the modus operandi of the X-ray. Perthes treated warts with suitable dosage, and removed them at different stages of the treatment. He found that all the cells of the papilloma, with the exception of the deep layers, underwent hornification. When properly treated, the cells of the germinal layer resumed their normal function, and proliferated, not to form a new papilloma, but new skin. It would seem from this experiment that the X-ray is capable of restoring to the cells their normal function. Those cells which were superfluous underwent hornification, which was their natural fate. The X-ray is found to act in a similar way upon cancer; the cells, however, having no useful function to perform, are apparently removed by the same processes which dispose of misplaced epithelium. Perthes states that an explanation of the activity of the X-ray upon warts is that it removes the stimulus which causes the cells of the deeper layer of the skin to proliferate beyond their normal boundaries.

Ehrlich's[2] observations on the development of sarcoma in the course of transplantation of carcinoma in mice appear to present further evidence of the existence of such a stimulus. Ehrlich's explanation of this phenomenon is, that, under given conditions, a stimulus present in the cancerous epithelium is transferred to the connective tissue of the stroma of the tumors, transforming them into sarcoma. There is no histological evidence of cytolytic action on the part of the immune forces in spontaneously recovered mice, and since the histological findings in tumors treated with the X-ray and those undergoing spontaneous recovery are identical, a plausible explanation is that both the X-ray and the immune forces in spontaneous retrogression act in opposition to some stimulus which causes the limitless proliferation of the cancer-cell.

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[1] Arch. f. Klin Chir. bd. lxxiv, h. 2.

[2] Berl. Klin. Wochenschr., 1905, No. 28.

CONCLUSIONS.

1. Spontaneous cure of mice successfully inoculated with the Jensen tumor occurs, in our experience, in about 23 per cent. of the animals.

2. The chances of spontaneous cure are inversely proportional to the size of the tumor. The frequency of the occurrence and its distribution in animals suggests that it may be more frequent in human beings than is generally supposed.

3. The occurrence of spontaneous recoveries from cancer, indicating the existence of immune forces capable of terminating the disease, demonstrates that cancer is not necessarily incurable, and should serve as an additional stimulus to research directed toward the development of a serum-therapeutic treatment.

APPENDIX

Besides the cases of spontaneous cure reported in the body of this article, all of which are accompanied by histological diagnoses, there have been reported numerous cases purporting to represent spontaneous cure. From the clinical history of some of these, it is highly probable that they are authentic cases, but as they are unconfirmed by histological examination, they are placed in an appendix for the interest which they may have in connection with the critical portion of our article. The literature furthermore contains frequent reports of the retrogression of metastases and interesting statistics relating to recurrence after incomplete operation. Spontaneous retrogression of metastases plays an important part in the history of chorion carcinoma.

The epitomized facts taken from Teacher of these cases, are as follows:

In Chroback's case, published in 1896, patient was 33 years old, had had two births and one abortion. Previous pregnancy was abortive and the onset was marked by a severe hemorrhage renewed five months later, rigor, hæmoptysis, foul discharge with fever. The treatment consisted of curettage, and hysterectomy eight or nine months after the onset. Patient recovered and has remained well so far as known. There were clinical evidences of metastasis in the lungs and extirpation was undertaken on clinical grounds in spite of the signs of metastasis.

Von Franqué's case was a woman 32 years of age who had had six pregnancies. Four weeks after the last one, which was perfectly normal, there was intermittent hemorrhage, weakness, hæmoptysis. Four months later curettage and four and one-half months later hysterectomy. She recovered rapidly and was well seventeen months later. The primary tumor was in the uterus with probable metastases in the lungs. Diagnosis of retained placenta four months. Uterine wall felt as thin as paper;

twelve days after clearing out the uterus was as big as ever. Recovery in spite of metastasis.

In the case described by Zagorjansky-Kissel the tumors were present in the vagina two weeks after an abortion in the third month. Diagnosis of primary vaginal tumor with sound uterus was made by Landau and Pick. Both nodules were excised and the uterus curetted. Nothing suspicious being found in the latter it was not removed. The patient's health improved immediately. She had had rigors and hæmoptysis, which after a few days ceased. The physical signs in the lungs were negative throughout. Eight months after the operation she had an abortion in the sixth week and at fifteen months she was three months pregnant and while not in the best of health showed no signs of recurrence of tumor.

Ladinski's case was a woman 19 years old, who had had one birth and one mole. Hemorrhage was continuous with the latter with cough and hæmoptysis; patient very ill. Abdominal hysterectomy was done nine weeks later, followed by recovery and patient was well eighteen months later. The primary tumor was in the uterus, small and deeply burrowing, containing villi. Symptoms indicating involvement of the lungs appeared during the pregnancy; disappeared after confinement, reappeared and finally disappeared after the hysterectomy. Recovery in spite of metastasis.

Kworostansky's case was a woman 24 years old who had had two births and one abortion; six months after the latter there was "menorrhagia," with severe bleeding, cough, tubercle bacilli in sputum. The treatment was curettage at six and seven months, hysterectomy at seven and one-half months. Patient was well in four and one-half months. Primary tumor was in the uterus with probable metastasis in the lungs. Cough disappeared as she got well. Recovery in spite of metastasis.

The case of Lonnberg and Mannheimer was that of a woman of 42, who had had two births and one mole. The last pregnancy, a mole, four months. Seven weeks following this there was bleeding and bloody discharge, pain in thorax and abdomen, anæmia. Two years later hysterectomy with removal of vaginal tumors. This was followed by recovery and the patient was well eighteen months later. The primary tumor was in the uterus with about twenty nodules "like varices" in vagina. It was a very slow case. Apparently metastasis in lungs at one year. Very much reduced but made rapid recovery. "Carcinoma syncytiale." Recovery in spite of metastasis.

The case of Pestalozza was a woman 22 years old, who had had one birth and one mole, two months. Following the latter there was anæmia, hemorrhage, cough and hæmoptysis. After three months hysterectomy was performed and patient was well one year later. The primary tumor was in the uterus with metastasis in the lungs(?). Diagnosed subinvolution and phthisis pulmonalis; no tubercle bacilli in sputum; curetted and found it was deciduoma. Recovery in spite of metastasis. That pulmonary metastases in chorion carcinoma are occasionally subject to spontaneous retrogression is proven by the observations of Risel, at the autopsy of a patient who had died of chorion carcinoma. Patient had suffered from hæmoptysis and coughing before death, the lungs containing plentiful metastases at the autopsy. The metastases in the lungs presented both macroscopic and microscopic evidences of spontaneous retrogression. The cells were atrophying and some regions showed areas of necrosis. The nodules were in a state of progressive organization from the periphery by connective tissue.

Authentic cases of retrogression of metastases of carcinoma other than chorion carcinoma may also be found in the literature. The cases reported by Osler are of interest.

Osler (" the Medical Aspects of Carcinoma of the Breast, with a note on the Spontaneous Disappearance of Secondary Growths," American Medicine, vol. i, 1901, p. 17) reports two cases in which there was apparent retrogression after well-defined metastasis accompanied by great general improvement in cases which had previously seemed hopeless. Short epitomes of the two cases are as follows:

Case 5. Tumor in right breast noticed January, 1897; operation, November, 1897, scirrhus; good health for a year; in September, 1898, pains in the back and down the legs; recurrence in the right eye; gradual increase of the pains in the legs; orthopnea;

emaciation; recurrence in the left breast; tumor mass on sternum; effusion in right pleura. After remaining for months in a desperate condition, gradual improvement; disappearance of tumor on sternum; improvement in vision of right eye; throughout 1900, persistence of pains to slight extent, but general improvement.

Case 6. Tumor of breast in 1896; operation by Dr. Tiffany, February, 1898; scirrhus; good health until August, 1899, when she had nerve-root pains and an attack of shingles; severe pains all winter; in March, 1900, complete paraplegia, with semiconscious condition, and the patient lay at death's door for days. Gradual improvement; disappearance of the paraplegia; slight recurrence in the scar; complete recovery of power in the legs; no disturbance of sensation; in fairly good health at the end of 1900.

It is evident in both of these cases there were metastases in the spinal region, clearly shown by the symptoms, which apparently underwent retrogression. In the first case quoted there was a visible tumor on the sternum which disappeared under observation.

The question of latency and freedom from recurrence after operation is intimately associated with spontaneous cure. In this connection the statistics collected by Shield are of great interest. Shield's statistics cover 108 cases, taken largely from the private practice of well-known surgeons. Many of these cases are unconfirmed by microscopic examination, but Shield assumes that the naked eye appearances of cancer of the breast are sufficiently familiar to competent surgeons to render the element of error small. He has divided the cases into two groups, the first containing forty-four, which were not followed by recurrence from a period of six to twenty years. They are as follows:

From 6 to 12 years, 23 cases.
From 12 to 20 years, 16 cases.
Twenty years and over, 5 cases.

Group 2 contains forty-nine cases in which recurrence took place. He emphasizes the fact that these cases must undoubtedly have been cancer.

RECURRENCE AFTER OPERATION WAS OBSERVED:

From the 4th to the 8th year in 19 cases
In the 9th year 8 cases.
In the 10th year 10 cases.
In the 11th year 2 cases.
In the 12th year 1 case.
In the 13th year 1 case.
In the 16th year 1 case.
In the 17th year 1 case.
In the 19th year 1 case.
In the 22nd year 2 cases.
In the 23rd year 1 case.
In the 25th year 2 cases.

In the fifteen remaining cases recurrences of a local nature in the scar or glands took place early, say within two years, and were removed with striking results.

Of twenty-three cases in which recurrences had been removed, sometimes repeatedly, we find —

4 cases remained well for 5–10 years.
11 cases remained well for 10–15 years.
3 cases remained well for 15–20 years.
4 cases remained well for 20–30 years.
1 (an extreme case) 49 years.

Pawlik, however, has reported the results in incomplete removal of epithelioma of the cervix by galvano-cauterization, in the clinic of C. V. Braun. One hundred and twenty-six of these patients withstood the operation. Twenty-nine of these cases were free from recurrence for periods varying from two to twenty years. The following is an analysis of the cases:

1 case still free after 20 years 11 months.
1 case still free after 18 years 2 months.
1 case still free after 17 years 4 months.
1 case still free after 14 years 3 months.
1 case still free after 11 years 5 months.
2 cases still free after 10 years 5 months.
2 cases still free after 8 years 8 months.
2 cases over 6 years.
2 cases over 5 years.
3 cases over 4 years.
8 cases over 3 years.
5 cases over 2 years.
—
29

In all of these cases a microscopic diagnosis was made and it is obvious that in a certain definite proportion of them the cauterization did not remove the entire tumor. The literature on this subject has been collected by R. Lomer (Zeitschr. f. Geburtshilfe u. Gynäkoligie Bd. L. H. 2). His article leaves absolutely no doubt that long periods of immunity from recurrence and in many cases ultimate cure, are obtained in cases in which all of the tumor has not been removed.

The following cases are without microscopical examination but appear to be clinically established:

A Case in which a Large Pyloric Tumor Disappeared after Gastro-Enterostomy. Post-Mortem Examination Eleven Years after Operation.

By Fred. Bowreman Jessett, F. R. C. S., England.

The patient, a widow, aged fifty-six years, was admitted to the Cancer Hospital, Brompton, February 24, 1890, suffering from pyloric obstruction. She had noticed a lump in her abdomen for over a year but had suffered for some time previously with chronic indigestion. No history of hæmatemesis. Vomiting present and increased gradually until November, 1899, at which time she was losing flesh rapidly and suffering intensely and was unable to keep anything on her stomach, usually vomiting within a few hours after eating. Tumor, which was situated in the epigastric region, gradually increased in size. On admission the tumor was found to occupy the whole epigastric region situated apparently over the pylorus and extending along the anterior wall of the stomach. It was movable and also moved on respiration. The stomach was enormously dilated, extending as low as the pubes. Patient vomited once or twice a day a brownish fermenting fluid filled with sarcinæ. She was very emaciated and weak. All the food given by the mouth caused pain, and shortly after, vomiting. The size and fixity of the tumor precluded pylorectomy and, in April, 1890, a gastro-jejunostomy was performed. At the operation the tumor was carefully examined and was found to be situated at the pylorus and had all the appearances of a scirrhus. It was adherent to the surrounding parts and was of the size of a small cocoanut. Patient left the hospital two months after the operation and has been seen periodically until November, 1900, when she died of apoplexy.

At the autopsy the stomach was found to occupy practically its normal position. It was constricted at the center forming a typical hour-glass contraction, the point of contraction being firmly adherent to the left lobe of the liver. The site of the operation was found to be on the anterior portion of the distal pouch. The duodenum was of normal caliber. On opening the distal pouch of the stomach the opening between it and the jejunum was found to admit two fingers. The constriction of the stomach had so reduced its lumen that an opening barely admitting one finger was left. Immediately behind this constriction was the pyloric opening into which a finger could be admitted with difficulty. The pylorus and constricted portion were hard and firmly adherent to the left lobe of the liver. This was the only remaining sign of the large growth found at the time of the operation. The stomach walls were normal; there was no thickening. The other organs were healthy.

A somewhat similar case is recorded by Dr. Demoulin and Dr. Tuffier. It was that of a naval lieutenant, aged 32, who suffered from severe dyspeptic symptoms early in 1897. In 1898 a hard swelling developed in the epigastrium; all medical treatment was of no avail and the patient became emanciated. The swelling was as large as an orange. Malignant tumor was diagnosed and Dr. Demoulin operated on December 1, 1899. A soft tumor was found situated around the pylorus and the adjacent parts of the stomach and the duodenum and it felt like a fatty growth; there were no enlarged glands. The patient was so exhausted that pylorectomy was considered impracticable. Dr. Demoulin, therefore, performed gastro-enterostomy. The patient made a slow recovery. On the twentieth day after the operation Dr. Demoulin palpated the epigastrium and to his surprise the tumor could no longer be felt, its site being marked by a little thickening which vanished within ten days. In May the patient was again on the active service.

The (so-called) Spontaneous Disappearance of Solid Abdominal Tumors, with three Cases.

By J. Greig Smith, *Proceedings of the Royal Medical and Chirurgical Society of London, Jan.* 21, 1894.

Case 1. A young man, aged 25. Intestinal obstruction existed due to a solid tumor as large as a cocoanut situated in the lower abdomen. The tumor being, on inspection, diagnosed as malignant, and being adherent to intestines, was not disturbed. Enterostomy was performed for the obstruction. The tumor slowly diminished in size and six months later when the abdomen was opened for the performance of enterorrhaphy it was found to have disappeared. The patient after four and one-half years continues in good health.

Case 2. A lady, aged 55. The tumor lay in the umbilical region and was adherent to and around the umbilicus. It was globular in shape and as large as a child's head at birth. On abdominal section the intestines were found to be adherent to its surface on one side. Diffusing malignancy was diagnosed and no attempt at removal was made. A small collection of muco-pus at the umbilicus was evacuated; a slight discharge from a fistula which formed there continued over two years. The tumor disappeared, the sinus closed and the patient is now quite well.

Case 3. A girl, aged 19. Here the tumor was as large as an eight months' pregnancy, filling and distending the upper and right abdomen but not dipping into the pelvis. On abdominal section the tumor was diagnosed as malignant and unremovable. A fæcal fistula formed; the tumor gradually diminished in size and ultimately disappeared. The intestinal opening was closed by operation and the patient is now in good health.

Vanishing Tumors.

By D'Arcy Powers, F. R. C. S., England, *Lancet, London*, 1899.

Case 3. A man, 21 years, admitted into Harley Ward with a tumor between the umbilicus and the pubes, and apparently attached to the pubes. It was three or four inches broad and about the size of a large cocoanut. The swelling could be felt per rectum as a rounded mass movable by the finger. The patient had been losing flesh lately but his temperature was normal and his bowels acted naturally, there was no blood in his motions, and no pain on defecation; nor was there anything abnormal discovered in his urine or in the act of micturition. An incision was carried into the abdomen through the linea alba when the tumor was found to be so fixed to the pelvis as to render its removal impossible. It was covered with large tortuous veins and was of a dark-red color with the consistence of a soft solid. The wound was closed and united by first intention, the patient making an uneventful recovery. He was discharged with the swelling apparently in the same condition as upon his admission to the hospital, or perhaps a little larger. Three months later he came back to the hospital and it was then found that the tumor had entirely vanished and that the patient had gained a stone and a half in weight.

Mr. Walsham, in commenting on this case, says very appositely that " it is of great interest as bearing upon the question of the occasional spontaneous disappearance of sarcomatous growths. It is of course possible that we were mistaken in our diagnosis and that the tumor was not sarcomatous. Its rapid growth, firm connection with the pelvic bones, progressive loss of weight and strength of the patient, taken with the appearance and consistency of the swelling itself, as revealed at the exploratory operation, led me to entertain no doubt as to its nature, and those of my colleagues who saw it with me were of the same opinion. This is the second case that I have personally met with of a supposed sarcoma in the abdomen disappearing spontaneously and several of my colleagues I know have had a similar experience. It may be added that there was no discharge of pus from the rectum, bladder, etc., nor any other sign of its having been an abscess or of an inflammatory nature; but of course one cannot lose sight of the possibility that it might have been an inflammatory or some other affection of the glands."

Case 4. A man, aged 54, was admitted on account of an abdominal tumor and vomiting, both of two months' duration. He had also been losing flesh and was constipated. A hard nodular mass as big as a man's fist could be felt in his abdomen, lying just below the liver and slightly to the right of the middle line. It was well-defined and was not particularly tender. An exploratory incision showed that the growth was adherent to the external abdominal wall in front, to the stomach and duodenum behind, and to the under surface of the liver above. The gall bladder seemed to be quite surrounded by the new growth, which thus proved irremovable and was thought to be malignant. The patient recovered rapidly from the operation and left the hospital three weeks afterward. He reported himself three months later to say that since the operation the swelling had "much dispersed." Four months afterward he again came to the hospital and was examined at "consulations" by many of the surgeons, who agreed unanimously that no tumor could any longer be felt in his abdomen, and at this time the man seemed to be in good health. But a few months later he was seen by Mr. Berry, the surgical registrar, in another hospital, suffering from jaundice and emaciation and there was but little doubt that he had malignant disease probably in or near the head of the pancreas, a diagnosis which was confirmed after the death of the patient.

Case 5. "A man was admitted into the Royal Free Hospital, under Dr. Seinsbury, and was seen by Mr. Boyce Barrow and myself (Mr. Berry). He had a rounded and hard lump as big as a fist in the region of the pylorus. We all thought it was malignant, although from its smoothness and some other features I never felt sure that it was not inflammatory. I opened the abdomen and found some adhesions which I broke down. The man made an excellent recovery and lost all his symptoms of pyloric obstruction. Nothing more was seen or heard of the lump after the operation."

Case 6. * * * The patient was a man, aged 24 years, who at the time of his admission to the hospital in March, 1890, gave the following history: About six years previously he received a blow on the top of his head from the flywheel of an engine. He took no notice of it at the time but a tumor began to grow a few months later at the place where he had been hurt. It reached the size of a sparrow's egg and then disappeared in about six months. Six or eight tumors subsequently appeared on different parts of the head and then diminished in size. Another tumor appeared in September, 1889, and grew steadily but quite painlessly until it was removed in March, 1890, when it measured ten inches in circumference. The scar caused by the removal of this tumor is seen in the model to lie over the left parietal bone. Microscopical examination showed the tumor to be a fibro-sarcoma, for it consisted for the most part of a round-celled sarcoma interspersed here and there with irregular connective tissue cells, and in places with bands of fibrous tissue.

Remarkable Disappearance of a Carcinomatous Tumor of the Throat.

TH. ZANGGER, *Korresp.—Bl. f. Schweizer Aerzte,* 19, 1904.

On January 5, 1904, Zangger was consulted by a decrepit frau who, on examination, proved to have a papillomatous tumor springing from the left side of the soft palate. In the course of the following three weeks the tumor grew to about twice the original size. Diagnosis of carcinoma was confirmed by several specialists but the patient refused operation. Tumor continued to grow until March, when it reached the size of an egg. Deglutition became more and more difficult and so painful that the patient had to be placed upon morphine and codeine to relieve insomnia. Patient at this time consented to an operation, but on further considering the case it was declared inoperable. Pronounced fetor, continuous salivation, and enlargement of the submaxillary glands on the left side were now observed. Milk could only be swallowed with the greatest pain and only when the head was held in a certain position. Patient remained in hospital until the beginning of June. Tumor presented all the characteristics of a malignant tumor. On the 2d of June the symptoms began to improve and from the 3d of June until the 13th of September there was no pain. An examination on the 4th of June, made by the nurse and confirmed by the patient, showed that the greater portion of the tumor had disappeared. An examination, made on the 2d of August and the 13th of September, showed a complete restoration of normal conditions in the throat with the exception of a few papillomatous growths on the left side of the soft palate varying in size from a pinhead to a small pea, and from this region running downward a definite scar. Enlargement of the submaxillary glands totally disappeared. General condition of the patient greatly improved. There is no record of microscopical examination in this case.

Observation VIII. (Service de M. le Dr. Vulpian, recueillie par M. Brunon interne da Service, in Gaz. des Hop. 1885.)

Eugenie, L.— Thirty-two years old, entered the clinic of Professor Vulpian at the Hôtel Dieu November 3, 1883.

About eighteen months ago patient noted that her left breast was painful to the pressure of her corset and that there existed under the skin a small tumor about the size of a hazelnut, which rolled under the finger. This tumor increased in size fairly rapidly and became adherent to the skin. Shooting pains appeared, only slight, situated all over the region, but without radiation in any special direction.

About ten months after its origin the tumor was about the size of a large egg. It began to ulcerate and there exuded a seropurulent liquid. The patient became very much weaker, then the tumor diminished in volume. The ulceration became cicatrized and finally the neoplasm became reduced to the size which it presented when she entered the hospital. Two months later, that is, one year after the beginning of the tumor in the left breast, the right breast became infected and has been the seat of the same condition. At this period the patient had reached a very advanced cachectic state. She took no nourishment, was without home, exposed to the cold, had persistent cough. She entered the Hôtel Dieu. She had scarcely the strength to walk. Frightfully thin, her complexion of a manifest straw color. She had a dry cough which was very fatiguing and moreover complained of sharp abdominal pains. On the whole she appeared in a state bordering on the last.

The tumors of the breast appeared as follows: The tumor of the left breast was about the size of an egg, but flattened out, hard and striated. It was adherent to the thoracic wall. There was no more movement of the integuments on the gland than there was of the gland on the thoracic wall. The skin in its neighborhood was gray and dull and rough like classical orange skin.

From the body of the tumor radiations extended irregularly toward the periphery, forcing themselves deeply in the tissue and encroaching on the skin. There was no longer any ulceration. On the right side the skin was adherent to the underlying tissues and the gland itself had not the hardness noted on the left side. In the left sub-clavicular triangle on the internal side of the two axillas one found lymphatic glands having the volume of a hazelnut, but rolling easily under the finger. In the supra-clavicular triangle were found masses not so well limited which were spread out and which were not painful on palpation. No pain in the arms but a fairly well-marked oedema of the whole arm. The hepatic region is sensitive to pressure and one finds deep in the hepatic region fairly well-marked elevation. The liver itself is increased in size. In the thickness of the abdominal wall one finds nodules of the size of a small nut, hard, flattened, giving the sensation of disks fixed in the interior of the integument. They number six or eight. On the thoracic wall in the region of the back of the neck on the posterior part of the body similar small tumors which are situated in the skin, which is, however, intact. There exists a slight exudation in the peritoneal cavity and in the left pleura, exudation which one estimates at about a liter or a liter and a half.

M. Vulpian, at the moment the patient is presented to him, does not hesitate to say that the prognosis is extremely serious, for there can be no doubt about the diagnosis having to do with an atrophied scirrhous tumor, accompanied by generalization. One can forsee the end is not far distant, seeing how serious is her general state. M. Vulpian prescribes a tonic treatment (potion de Todd, extract of cinchona, followed by syrup of iodide of iron and arsenate of soda).

November 5. The patient begins to take some nourishment. She still suffers from insomnia, but the abdominal pains and the cough are less marked.

November 12. Patient is able to get up for the first time.

December 1. One notes that the tumor in the left breast has begun to diminish in size, there is no longer the oedema of the arm nor of the ankles or appreciable ascites.

December 26. The patient having completely recovered her appetite and some color in her complexion, is sent to the Vêsinet.

January, 1884. On return from the Vêsinet one notes that her general condition has continued to improve. Patient says that she has never felt better in her life.

The left tumor no longer protrudes. The nipple is completely retracted. The tumor, as a whole, appears under the form of a flattened disk, the thickness of two centimeters, and gives a striated sensation. The smaller abdominal and dorsal tumors no longer exist. It is the same with the axillary lymphatics. At the base of the right lung a marked dullness still exists. Local vibrations are absent. The voice is a little vibrating.

January 25. The patient leaves the hospital finally finding herself in a state of health equal to that in which she was before these tumors appeared.

On the Influence Exerted on the Virulence of Carcinoma in Mice by Subjecting the Tumor Materials to Incubation Previous to Inoculation.

By George H. A. Clowes and F. W. Baeslack.

It is proposed to present in this communication the results of experiments made with a view to influencing the virulence of tumor cells by incubation previous to inoculation. The Jensen mouse carcinoma has been employed exclusively in this series of experiments, and, in view of the extensive literature on the subject, there is no need of further description of the tumor.

Jensen,[1] Loeb, Sticker, and Haaland have all, in the course of their transplantation experiments with animal tumors, determined the point at which the tumor materials, through exposure to high temperature, previous to inoculation, were rendered incapable of further proliferation. Jensen, working with his mouse carcinoma, found that exposure of the tumor materials suspended in normal salt solution (0.8 per cent.) to temperatures of 47° C. and upwards for a period of five minutes entirely destroyed the tumor cells, whilst at a temperature of 46° C. a distinct diminution in the yield of tumors, as compared with that obtained at lower temperatures, was observed. The number of animals employed in this experiment was extremely small, no control was made at normal temperatures, and no statements were made regarding the relative speed at which individual tumors in the different series developed.

Loeb[2] observed that exposure of the rat sarcoma, with which he was working, to temperatures of 45° C. and upwards for periods of thirty minutes or more, was sufficient to prevent further development on transplantation. In one case, employing materials which had been incubated forty minutes at 43° C., a fairly good yield was obtained (four fatal tumors in seven rats). He found, however, in those cases in which temperatures approaching 43° and 44° C. had been used, that the tumors made their appearance later than in the control series and that their speed of development was markedly retarded.

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[1] Jensen, *Centralblatt f. Bakteriologie*, Abt. I, Orig., 1903, xxxiv, 122.

[2] Loeb, *Virchow's Archiv*, 1903, clxxii, 345.

Sticker,[3] in an extensive series of experiments on the transplantation of a lymphosarcoma in dogs, made a single experiment in which small pieces of the tumor were subjected to a temperature of 50° C. for two hours previous to their introduction subcutaneously into a two-months-old puppy. Unfortunately the dog died of pneumonia ten days after the commencement of the experiment, but examination showed tumors to be developing at both points of inoculation, indicating that lymphosarcoma of this type was capable of resisting much higher temperatures than were the materials employed in the experiments recorded above.

Haaland,[4] in a recent communication from Ehrlich's laboratory, published the results of a series of experiments carried out on a mixed mouse tumor with a view to obtaining a sarcoma free from carcinoma cells. The tumor materials were introduced into thin-walled capillary tubes which were heated in a water-bath at a temperature of 44° C., for periods of five, ten, and fifteen, up to sixty-five minutes respectively. In a control series of six mice which had received normal materials without previous incubation, 80 per cent. yielded rapidly-growing tumors, whilst in the heated series the yield of tumors and the speed of development both diminished with the increasing period for which the materials had been incubated previous to inoculation. It was also observed that the carcinoma cells succumbed earlier than the sarcoma cells, with the result that materials which had been treated for thirty-five minutes and upwards yielded pure sarcomata in which no trace of carcinoma was to be found. Haaland considered that the reduction in virulence and speed of development was not attributable solely to the destruction of a large proportion of the cells, but rather to some deep-seated change in the cell mechanism, indicated by the histological findings.

It will be observed that all these authors made use of relatively high temperatures and for the most part of fairly virulent materials, confining their attention principally to a determination of the point at which the cells employed were no longer capable of proliferation. From the statements of Loeb and Haaland, it is apparent that not only was the number of tumors in parallel series decreased with increasing temperatures to which the materials had been exposed previous to inoculation, but, further, a distinct retardation in the development of individual tumors of the high-temperature series was noted by both observers.

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[3] Sticker, *Zeitschrift f. Krebsforschung*, 1904, i, 413.

[4] Haaland. *Berl. klin. Wochenschr.*, 1906, xliii, 40.

All those who have carried on extensive experiments on the transplantation of animal tumors are aware of the extraordinary variations which occur in one and the same series, both in the percentage of tumors and in the speed of development of individual tumors of the series, all of which might be attributable to a variation in the concentration of a stimulating agent causing proliferation of the cancer cells.

Our experiments started with the conception that exposure to temperatures slightly above the normal for short periods of time might exert an effect upon weak and attenuated tumor cells, stimulating them to more active development on subsequent inoculation in a manner analogous to the action of poisons at high dilution on yeasts, bacteria, etc. The activity of catalytic chemical agents on enzymes, toxins, etc., has been demonstrated to increase with increasing temperatures, and since the stimulating effect of poisons is exerted only within very narrow limits of concentration, it is quite conceivable that a slight rise in temperature might so increase the activity of a stimulating body already present as to lead to a marked increase in the proliferative energy of the cell.

Before commencing the series of experiments in which it was necessary to make relatively accurate comparisons between the results obtained in experimental series and their controls, it was necessary to eliminate as far as possible all sources of experimental error calculated to introduce accidental variations. Our general procedure when inoculating mice, both in these and other experimental series, was as follows: a mouse having a suitable tumor was killed, the tumor removed and weighed and ground in a sterile mortar with three or four times its volume of salt solution. All coarse particles which might interfere with the uniformity of the mixture were removed by means of rakes and in some cases by filtration through coarse gauze, a procedure which, whilst calculated to afford a perfectly homogeneous mixture for experimental work, would also tend to reduce the percentage of tumors. The contents of the mortar were then measured and divided into three or four equal parts in sterilized test tubes. One portion was reserved for purposes of control, the remaining tubes being subjected to the required temperatures for a definite period of time in the Ostwald water-bath. After the completion of the incubation process, the contents of each test tube were introduced into a fresh sterile mortar, once more thoroughly admixed, and uniform doses, usually from 0.4 to 0.5 cubic centimeter of the mixture injected subcutaneously into each mouse of the series. The greatest care was observed in carrying out the series of experiments that the mice selected were all derived from the same source and approximately the same age, so that, employing, for example, ten mice for the normal materials and ten for those which had been incubated at each temperature, it might be said that the only varying factor introduced into these experiments was the influence exerted on the cancer material by subjecting it to incubation previous to inoculation.[5]

The mere enumeration of the tumors obtained would of course afford but a very inadequate idea of the relative virulence of tumor materials employed for the inoculation of comparative series. We were led, therefore, early in the course of our experimental work to adopt a system of identifying individual members of a group and following the development of their tumors at short intervals of time from inoculation to death. For this purpose chart books were employed in which the groups were arranged numerically and the individual members of a group alphabetically. For each individual mouse a series of diagrams was prepared showing the fore legs, hind legs, nose, and tail. When a tumor made its appearance, it was reproduced in the location in which it occurred, in a diagram as nearly life size as possible, and as the tumor increased in bulk and secondary tumors made their appearance, further chartings were prepared showing the progress of the growth until the death of the animal occurred or spontaneonus recovery intervened. (Gaylord and Clowes, On Spontaneous Cure of Cancer, *Surgery, Gynæcology, and Obstetrics,* 1906, ii, 633.)

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[5] As a control on the incubation experiments in this paper, the results obtained by inoculating two or more lots of mice on the same day with the same normal materials are of value. Without observing as extensive precautions as in the experimental series, the yields obtained in various comparable groups were so nearly uniform as to make it extremely probable that the results observed in the incubated series were attributable to the experimental treatment and not to accidental distribution. For example, lots 7 *a* and 7 *b*, inoculated June 10, 1904, with the same material, yielded, respectively, 4 tumors in 13 mice and 4 tumors in 11 mice. Lots 161 and 162 each of ten mice, inoculated December 21, gave 3 large tumors apiece. In other cases a difference of one or at most two tumors is observed, but in no normal cases are the differences in the yields as great as those recorded in the incubated series dealt with subsequently in this paper, in spite of the fact that practically no precautions were observed to insure uniformity.

Making use of such a system, it was possible to determine not only the actual number of fatal tumors in each group, but also the relative speed of development of individual tumors in the series, as indicated by the number of days from inoculation required to reach a certain size, and the volume of the tumors after a given number of days.

It would of course be preferable in a publication of this nature to include the complete charted records of every series of experiments, but since, on account of space limitations, this is impossible, reference has been made to the records wherever necessary in order to bring out the essential points.

The greatest variation in the virulence of tumors is frequently observed in the same series. The age of a tumor, that is to say, the period which has elapsed between the date of inoculation and the date on which the tumor has attained a size suitable for transplantation, frequently bears a marked relation to the yield of tumors obtained in the following generation. An illustration of this form of variation is to be found in the following table, in which are compared the yields obtained on further transplantation from a series of six tumors, all of which belonged to the same group, having been inoculated on the same day with the same materials:

Tumor.	Age in days.	No. mice employed.	No. of tumors.	Percentage of tumors.
214 A	45	19	8	42.0
216 B	56	7	2	28.5
213 B	59	38	11	29.0
214 B	66	36	10	28.0
Total 66 days and under		100	31	31.0
218 E	184	26	5	19.2
215 B	184	32	2	6.0
Total 184 days		58	7	12.0

It will be seen that the yield has fallen from 31 per cent. in the rapidly-growing tumors to 12 per cent. in those which required upwards of six months for development.

It has also been noted that very young tumors weighing less than four grams seldom give good results on transplantation. In fact, the best yields are obtained, as a rule, from tumors that are still developing fairly actively and weigh over five grams.

After preliminary experiments from which it was concluded, that no tumors could be obtained from materials incubated at temperatures above 44° C. for thirty minutes or more, we commenced the following series of experiments:

Experiment I.[6] November 19, 1904.

Mouse tumor 105 C employed for inoculation. Weight of mouse, 18.5 grams. Weight of tumor, 6.5 grams.

Age of tumor (time elapsed from date of inoculation to November 19), 40 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

Serial No.	Lot 142	Lot 143	Lot 144
Number of mice	8	8	8
Incubation of tumor materials previous to inoculation	Nil	30 mins. at 40°C.	30 mins. at 45°C.
Examination after 3 mos.	6 large tumors	1 large tumor	Nil

In the above series of experiments the tumor employed for inoculation was derived from an extremely virulent strain and incubation at 40° C. was found to lower the yield of tumors from 75 to 12.5 per cent.[7]

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[6] In order to avoid confusion, we have tabulated the results as far as possible. Under the lot numbers of comparable experimental series are found first the number of mice employed, followed by the treatment to which the tumor materials had been submitted previous to inoculation, and the results of examination of such series after a period of eight months.

Experiment II. December 5, 1904.

Mouse tumor 101 D employed. Weight of mouse, 22.5 grams. Weight of tumor, 3.5 grams.

Age of tumor, inoculation to death, 66 days.

First appearance of tumor to death, 28 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

Serial No.	Lot 119	Lot 150	Lot 151
Number of mice	8	8	8
Incubation of tumor materials previous to inoculation	Nil	10 mins. at 35°C.	10 mins. at 40°C.
Examination after 3 mos.	4 large tumors	2 tumors 1 spon. recovery	1 large tumor

The tumor employed in this experiment was also derived from a virulent strain and although small it developed rapidly in the course of a few days preceding the date on which the animal was killed. It was moderately necrotic and from its appearance was expected to yield a fair proportion of tumors. As has been seen from the table, the yield decreased with increasing temperatures to which the materials were submitted previous to inoculation.

Experiment III. March 17, 1905.

Mouse tumor 251 E employed. Origin, 142–105.[8] Weight of mouse, 52.5 grams. Weight of tumor 6.2 grams.

Age of tumor, inoculation to death, 53 days.

From first appearance of tumor to death, 36 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

Serial No.	Lot 166	Lot 174	Lot 190
Number of mice	9	9	6
Incubation of tumor materials previous to inoculation	Nil	15 min. at 40°C.	10 min. at 45°C
Period after inoculation.		Record of results.	
10 days.	2 small tum.	6 small tum.	Nil
15 days.	2 of 5 mm. diam. 2 of 3 mm. diam.	3 of 10 mm. diam. 3 of 6 mm. diam.	Nil
31 days.	2 of 10 mm. diam. 2 of 5 mm. diam.	1 death of tum. 4 lg. 1 sm. tum.	Nil
Final results, 4 months.	3 large tum. 1 small tum.	5 large tum. 1 small tum.	Nil

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[7] The percentage terms used in this case are simply for purposes of comparison, as it would obviously be impossible to make any calculations based upon such a small number of animals.

[8] In this and subsequent cases in which the origin of the tumor is given its direct descent is traced to one of the four groups, 101, 102, 105, 106, all of

As a final result it will be seen that five large tumors and one small tumor occurred in the incubated series in comparison with three large and one small in the normal series. In addition, it should be noted that the speed of development of the tumors in Lot 174 was far greater than that of the tumors of Lot 166. Fifteen days after inoculation the former were as far advanced as were the latter at the end of thirty-one days. Lot 174 afforded us a remarkable illustration of extremely rapidly developing tumors which became necrotic and were suitable for transplantation at a much earlier date than was normally found to be the case.

Experiment IV. April 7, 1905.

Mouse tumor 198 B employed. Origin, 150 B–101. Weight of mouse, 27.4 grams. Weight of tumor, 6.6 grams.

Age of tumor, inoculation to death, 38 days.

First appearance of tumor to death, 27 days.

Ratio of tumor to salt solution, 1 : 3.

Materials filtered through gauze and consequently considerably attenuated. Dose, 0.4 c.c.

Serial No.	Lot 227	Lot 224	Lot 225	Lot 226
Number of mice	6	6	6	6
Incubation	Nil	30 min. at 40°C.	2 hr. at 40°C.	19 hr. at 40°C.
Examination after 4 mos.	No tumors	1 tumor	1 tumor	No tumors

The low yield of tumors in this series is probably attributable in part to the filtration through gauze, but it should also be noted that the tumor in question was young and in active development and showed very limited areas of necrosis.

Experiment V. April 19, 1905.

Mouse tumor 41 B employed. Origin of tumor, 147–105. Weight of mouse, 30.5 grams. Weight of tumor, 5.2 grams.

Age of tumor, inoculation to death, 62 days.

First appearance of tumor to death, 40 days.

Ratio of tumor to salt solution, 1 : 4.

Material filtered through gauze. Dose, 0.4 c.c.

Serial No.	Lot 234	Lot 235
Number of mice	6	6
Incubation	Nil	1 hour at 41°C.
Examination after 6 mos.	1 slow-growing tumor weighing 1 to 2 grams	4 large rapidly growing tumors weighing 5 to 9 grams.

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which were in the second generation from the original Jensen tumor. For family tree showing relationship of individual groups see *American Journal of Physiology*, 1905, xiv, 177.

Experiment VI. April 19, 1905.

Mouse tumor 199 F employed. Origin, 150 B–101. Weight of mouse, 24.2 grams. Weight of tumor, 9.3 grams.

Age of tumor, inoculation to death, 50 days.

First appearance of tumor to death, 38 days.

Ratio of tumor to salt solution, 1:4.

Material filtered through gauze. Dose, 0.4 c.c.

Serial No.	Lot 236	Lot 237
Number of mice	7	6
Incubation	Nil	1 hour at 41°C.
Examination after 4 mos.	1 slow-growing tumor	1 rapidly growing tumor.

The extreme attenuation in this group resulting in the production of one tumor only in each of the series is probably attributable to the filtration through gauze.

The four preceding series are remarkable in that in no case is there found to be a smaller yield of tumors in the groups incubated at 40° or 41° C. than in those which received no treatment. Summarizing the results it may be said that whilst six slow-growing tumors were obtained from thirty mice in the normal series, ten rapidly growing and two slow-growing tumors, making a total of twelve tumors, were obtained from twenty-nine mice in the groups incubated at 40° or 41° C. If we exclude the two series in which filtration through gauze has led to such a marked attenuation that four tumors only were obtained from the inoculation of thirty-nine mice, we find that in the two remaining series five slow-growing tumors were obtained from fifteen mice in the normal groups, as against eight fast- and one slow-growing tumors out of fifteen mice in those groups which had received injections of tumor materials previously incubated at 40° or 41° C.

Having thus demonstrated that incubation at relatively high temperatures is not necessarily prejudicial, and, in the case of those tumors which afforded a small yield at normal temperatures, actually led to an increase, we determined to employ tumors known to possess a high degree of virulence. For this purpose two tumors of Lot 174 were chosen for the following experiments, VII and VIII. This group, referred to above in Experiment III, was characterized by the extreme rapidity with which the tumors of its members reached maturity, as indicated by a peculiar type of noninfected necrosis, which has been found on experiment to yield the maximum of tumors on transplantation.

Experiment VII. May 5, 1905.

Mouse tumor 174 G employed. Origin, 251 E–142–105. Weight of mouse, 22.0 grams. Weight of tumor, 5.2 grams.

Age of tumor, inoculation to death, 49 days.

First appearance of tumor to death, 35 days.

Tumor partially necrotic but not infected.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

Serial No.	321	322	323	324
Number of mice	8	8	8	8
Incub. of tum. mat. previous to inoc.	Nil	1 hr. at 40°C.	1 hr. at 42°C.	1 hr. at 44°C.
Period after inoculation		Record of results		
24 days.	4 large tum. 2 small tum.	1 large tum. 3 small tum.	Nil	1 small tum.
Final results after 4 mos.	6 large tum. 1 intermed. t.	3 large tum. 1 small tum.	1 small tum.	1 large tum.

It will thus be seen, to summarize the results obtained by making use of this extremely virulent material, that employing eight mice in each case, seven tumors were obtained in the normal series, four in the series incubated at 40° C., and one each in the series incubated at 42° and 44° C.

Experiment VIII. May 16, 1905.

Mouse tumor 174 B employed. Origin, 251 E–142–105. Weight of mouse, 24.8 grams. Weight of tumor, 4.3 grams.

Age of tumor, inoculation to death, 60 days.

First appearance of tumor to death, 50 days.

Tumor partially necrotic but not infected.

Ratio of tumor to salt solution, 1:4.5. Dose, 0.5 c.c.

Serial No.	329	330	331	332
Number of mice	8	8	8	8
Incub. of tum. mat. previous to inoc.	Nil	1 hr. at 39°C.	1 hr. at 41°C.	1 hr. at 43°C.
Period after inoc.		Record of results		
28 days	1 large, 2 mod. and 1 small tum.	1 mod. tumor	1 mod. tumor	Nil
Final results, 4 months	5 large tumors	3 large tumors	2 large tumors	Nil

To summarize the results it may be said that there developed in the normal series five tumors, in the series incubated at 39° C. three tumors, in that at 41° C. two tumors, and at 43° C. nil.

In the two preceding series, VII and VIII, we were dealing, as in Experiments I and II, with materials above the normal average of virulence and in both cases incubation appears to diminish the proportion of successful transplantations, and, further, to effect a slight retardation in the speed of development as compared with the normal.

On May 19 and 23, two series of experiments were carried out in which the materials were incubated two hours at 43° to 44° C. previous to inoculation. This treatment produced such a marked inhibition, only one tumor being obtained in the entire series, that we have excluded the experiment in question and must assume that such a prolonged treatment at a temperature as high as 44° C. is capable of entirely destroying the tumor cells, thus rendering further proliferation impossible.

An analysis of the results obtained in the preceding eight series made it appear probable that whilst treatment at a temperature of 38° to 41° C. was distinctly unfavorable to the development of virulent tumors, the same process produced a stimulating effect leading to an increased proportion of tumors as compared with the normal when nonvirulent materials were employed.

In the six remaining series an attempt was made to compare the results of slowly and rapidly growing tumors, of small and large tumors, etc.

Experiment IX. October 11, 1905.

Mouse tumor 340 A employed. Origin, 213 B–175–161–128–101. Weight of mouse, 32.75 grams. Weight of tumor, 6.4 grams.

Age of tumor, inoculation to death, 138 days.

First appearance of tumor to death, 119 days.

Tumor only slightly necrotic.

Ratio of tumor to salt solution, 1:3. Dose, 0.6 c.c.

Serial No.	469	470	471
Number of mice	8	8	8
Incub. of tum. mat. previous to inoc.	Nil	30 min. at 38.5°C.	30 min. at 42.2°C.
Period after inoc.		Record of results	
41 days	1 mod. tumor	2 mod. tumors	
	1 small tumor	2 small tumors	2 small tumors
58 days	1 mod. tumor	2 large tumors	
	1 small tumor	2 small tumors	1 small tumor
Final results, 4½ months	2 mod. tumors	2 large tumors	
		1 small tumor	Nil

Experiment X. October 11, 1905.

Mouse tumor 427 A employed. Origin, 395 C–232 B–195 D–169 C–106.

Weight of mouse, 37.9 grams. Weight of tumor, 9.9 grams.

Age of tumor, inoculation to death, 43 days.

First appearance of tumor to death, 30 days.
Tumor far more necrotic than 340 A used in Experiment IX.
Ratio of tumor to salt solution, 1 : 3. Dose, 0.7 c.c.

Serial No.	472	473	474
Number of mice	10	10	10
Incub. of tum. mat. previous to inoc.	Nil	30 min. at 38.5°C.	30 min. at 42.2°C.
Period after inoc.		Record of results	
41 days	4 mod. tumors 2 small tumors	2 mod. tumors 3 small tumors	Nil
58 days	4 large tumors 2 mod. tumors	1 large tumor 1 mod. tumor	Nil
Final results, 4½ months	6 large tumors	1 large tumor 1 mod. tumor	Nil

The above results obtained in Experiments IX and X are interesting in view of the fact that they afford us a comparison between two tumors which showed a great difference in their speed of development. 340 A, used in Experiment IX, was inoculated on May 26, showed a tumor as early as June 14, which increased in size until June 26, on which date it diminished and remained stationary for a couple of months, finally, about the beginning of September, developing once more until on October 11 it had reached a sufficient size to be used for transplantation. Mouse No. 427 A, used in Experiment X, was inoculated August 29 and its tumor had reached considerable dimensions by September 18 and as stated above weighed almost ten grams on October 11. Thus the tumor which required 138 days to reach 6.4 grams showed practically uniform results in the three series of experiments, an actual increase in the number of tumors being noted at a temperature of 38.5° C., whilst the tumor which required only forty-three days to reach a weight of 9.9 grams and showed marked necrosis, gave a large yield, 66 per cent., at normal temperatures falling to 22 per cent. at 38.5° C., and to nothing at 42° C.

Experience had shown that small, hard tumors gave extremely poor yields on transplantation. We determined, therefore, to make use of two tumors, both of which had been inoculated on the same day, one of which had grown slowly and the other rapidly.

Experiment XI. October 25, 1904.

Mouse tumor 429 A employed. Origin, 328 C–214 A–175 C–161 M–128–101. Weight of mouse, 24.7 grams. Weight of tumor, 4.2 grams.

Age of tumor, inoculation to death, 48 days.

First appearance to death, 30 days.

Tumor small, hard, and not necrotic.

Ratio of tumor to salt solution, 1: 3. Dose, 0.5 c.c.

Serial No.	505	506	507
Number of mice	10	10	10
Incub. of tum. mat. previous to inoc.	Nil	20 min. at 36°C.	20 min. at 40.5°C.
Period after inoc.		Record of results	
27 days	3 small tumors	2 mod. tumors 4 small tumors	1 large, 1 mod., and 5 small tum.
44 days	2 small tumors	3 large tumors 3 med. tumors	4 large, 1 mod., and 2 small tum.
Final results, 4 months	1 small tumor	5 large tumors 1 med. tumor	4 large tumors 1 med. tumor

Experiment XII. October 25, 1904.

Mouse tumor 432 B employed. Origin, 329 E–174 B–100 E–142–105. Weight of mouse, 36.2 grams. Weight of tumor, 11.8 grams.

Age of tumor, inoculation to death, 48 days.

First appearance of tumor to death, 25 days.

Tumor large, soft, cystic, and necrotic but not infected.

Ratio of tumor to salt solution, 1:3. Dose, 0.5 c.c.

Serial No.	508	509	510
Number of mice	10	10	10
Incub. of tum. mat. previous to inoc.	Nil	20 min. at 36°C.	20 min. at 40.5°C.
Period after inoc.		Record of results	
27 days	2 med. tumors 1 small tumor	1 med. tumor 2 small tumors	1 med. tumor 1 small tumor
44 days	2 large tumors 2 med. tumors	1 large tumor 3 med. tumors	1 large tumor
Final results after 4 months	2 large tumors 2 med. tumors	4 large tumors	1 large tumor

Comparing the results of the two preceding experiments it will be seen that whilst the incubation of the small, hard tumor led to a great increase, from 10 to 60 per cent., in the yield of tumors in the succeeding generation, the incubation of the large, soft, cystic tumor produced relatively less effect, a decrease in the proportion of tumors at 40° C. being noted, as compared with the normal.

The final series of incubation experiments was carried out making use of two slow-growing tumors both of which had shown at some stage more or less tendency to remain stationary or retrogress.

Experiment XIII. December 12, 1904.
Mouse tumor 419 B employed. Origin, 383 A–304, A–200, A–150, B–101.
Weight of mouse, 18.8 grams. Weight of tumor, 2.9 grams.
Age of tumor, inoculation to death, 112 days.
First appearance of tumor to death, 80 days.
Ratio of tumor to salt solution, 1:4. Dose, 0.4 c.c.

Serial No.	590	591	592
Number of mice	8	8	8
Incub. of tum. mat. previous to inoc.	Nil	30 min. at 37°C.	30 min. at 40°C.
Period after inoc.		Record of results	
Final results after 10 weeks	2 large tumors	4 large tumors 1 mod. tumor	1 large tumor 1 small tumor

Experiment XIV. December 12, 1904.
Mouse tumor 462 C employed. Origin, 218 E–175, C–161, M–128–101.
Weight of mouse, 33.6 grams. Weight of tumor, 5.3 grams.
Age of tumor, inoculation to death, 74 days.
First appearance of tumor to death, 40 days.
Ratio of tumor to salt solution, 1:3. Dose, 0.5 c.c.

Serial No.	593	594	595
Number of mice	10	9	10
Incub. of tum. mat. previous to inoc.	Nil	30 min. at 37°C.	30 min. at 40°C.
Period after inoc.		Record of results	
Final results after 10 weeks	2 large tumors	Nil	4 large tumors

These two series show, as would be expected, a rather larger proportion of tumors in the incubated than in the normal series; whilst 419 B shows a maximum at 37° C., 462 C gives the highest yield from materials incubated at 40° C. The complete absence of tumors in lot 594 is extremely difficult to explain, and forms the most serious exception in the entire series of experiments enumerated above. We have endeavored to find some explanation for this discrepancy. The ulceration of tumor materials in some mice of this series would indicate a possible contamination during the course of the incubation process. We are not, however, aware of any such accident having occurred, and must, therefore, conclude that in spite of the utmost precautions, discrepancies will occur when dealing with materials of this nature.

Excluding the two series of experiments in which filtration through gauze or exposure to temperatures above 44° C. for more than an hour would materially interfere with results, we find that twelve series of experiments have been carried out employing thirty-seven lots of mice, each of which contained from six to ten individuals.

We have included two tables in which the results obtained after three months by incubating five strong and five weak strains at 40° to 41° C. are collected. In the first column is given the number of the mouse employed originally for inoculation of the series. In the second column is the number of mice employed in each series, both for the normal and incubated material. In the third column is found the number of tumors present in the normal series, the number preceding the + sign representing the rapidly-growing tumors and that following, the slowly-growing tumors. In the final column is found the number of rapidly and slowly-growing tumors in the incubated series.

TABLE I. VIRULENT GROUPS.

Mouse.	Number of mice in each series.	Tumors of normal series.	Tumors of incubated series.
105 C	8	6 + 0	1 + 0
101 D	8	4 + 0	1 + 0
174 G	8	6 + 1	3 + 1
174 B	8	4 + 1	1 + 1
432 B	8	2 + 2	1 + 0
Total	40	22 + 4	7 + 2
		55% + 10% = 65%	17.5% + 5% = 22.5%

TABLE II. NONVIRULENT GROUPS.

251	9	3 + 1	5 + 1
41 B	6	0 + 1	4 + 0
429 A	7	0 + 1	4 + 1
419 B[9]	8	2 + 0	1 + 1
462 C	10	2 + 0	4 + 0
Total	40	7 + 3	18 + 3
		17.5% + 7.5% = 25%	45% + 7.5% = 52.5%

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[9] 419 B gave a maximum yield of 50% on incubation at 37° C. and presumably was already suffering attenuation when heated to 41° C.

In some cases the maximum is obtained at 37° or 38° C., a fall already being noticed at 40° and 41° C. Temperatures of 42° C. and upwards seem in all cases to exert an inhibiting effect upon the tumor development.

An inspection of the above table shows that by incubating the tumor materials in salt solution for varying periods of time at a temperature equal to the maximum fever temperature, a greatly increased yield was obtained from relatively weak strains, the total number of tumors being more than twice as great in the same number of mice. On the other hand, incubation at the same temperature produced a marked inhibiting effect where highly virulent materials were employed, a fall to less than one-third of the number of rapidly-growing tumors and from 65 to 22.5 per cent. in the total yield being recorded.

Finally, reference should be made to practical application of incubation as a means of stimulating proliferation of tumors. The transplantation of a primary mouse carcinoma presents far greater difficulties than does the reinoculation of such a tumor in subsequent generations. Any means, therefore, of stimulating the cancer cells to more active proliferation must possess considerable value from an experimental standpoint. Several mice having primary carcinoma similar to that of the Jensen mouse have been sent to the laboratory from a common source in the course of a few months. All attempts which were made to transplant the carcinomatous tissue by ordinary means having failed, it was decided to make an attempt to increase the virulence of any subsequent tumor of this group by incubation previous to inoculation. This method afforded extremely satisfactory results, a considerable tumor being obtained in a mouse inoculated with materials which had previously been incubated twenty minutes at 39° C. This tumor was incubated and transplanted, and three tumors were obtained in the following generation. The tumor has now been transplanted through three generations and may, therefore, be looked upon as successfully established.

The most remarkable facts in connection with this experiment are:

1. Whilst twenty-seven mice which have received incubated material have yielded eight tumors, fifteen mice which received identically similar materials, but without incubation previous to inoculation, have yielded no tumors.

2. The virulence of the tumor has increased in the course of three generations to a marked degree. Whilst ten weeks were required for the development of the first tumor, and five weeks for those in the second generation, tumors of considerable size have already made their appearance in the third generation in less than a week.

We do not attribute any special significance to a single experiment of this nature, but since it appears probable that incubation was instrumental in establishing this series, we are making use of incubation for half an hour at temperatures of 38.5° to 39.5° C. in all cases in which primary tumors are sent to the laboratory, and have included a note on the subject in the hope that our method may possibly prove of value to others working in the same field.

SUMMARY AND CONCLUSIONS

Our experience with mouse tumors has demonstrated that a great difference exists not only in the relative immunity possessed by different individuals but also in the relative virulence of different tumors all owing their origin to the same source.

In this paper it has been shown that the variations in question can be accentuated by incubation of the transplantation materials previous to inoculation at temperatures varying from 37° to 41° C. In the case of extremely virulent tumors giving a yield of upwards of 60 per cent. under normal conditions, a distinct and gradual fall in virulence accompanies increasing temperatures of incubation until a point is reached at which no further tumors are obtained. On the other hand, relatively inactive materials giving comparatively small yields of tumors under normal conditions may be so stimulated by subjecting them to incubation at suitable temperatures as to afford a larger yield and relatively more rapidly-growing tumors than were obtained in the normal series. In these cases the optimum temperature (that is to say, the temperature at which when incubated for a definite period of time, the materials employed yield the largest proportion of tumors) appears to vary, for whilst the most virulent materials, as stated above, exhibit an optimum yield at normal temperatures decreasing with incubation, less virulent materials show an optimum at temperatures slightly above the normal, whilst the least virulent of all show an optimum at as high a temperature as 40° to 41° C., all the curves eventually falling to zero, as those temperatures are reached at which the cells themselves might be expected to succumb.

This fact having been demonstrated, it remains to compare the histories of the various tumors employed for the sixteen series of experiments. The charted diagrams representing the course of development of each individual tumor from the date of inoculation to the date of death have been prepared and afford an admirable means of comparison of the relative speed of development of the tumors employed and consequently of their relative proliferative energy. The origin of all tumors for several generations, whether from a relatively active or inactive stock, may be determined from the records, and data regarding the age, weight, and pathological state of the tumor are also available.

1. Those tumors which give a small yield under normal conditions, but which give an increased yield on incubation, are either rapidly-growing young tumors, or small and hard tumors, or have been extremely slow in their development, and have in certain cases exhibited indications of a tendency to undergo spontaneous recovery. Tumors of this class seldom exhibit any marked indications of necrosis of the type which will be referred to in the next paragraph.

2. Those tumors which on inoculation give a large yield at normal temperatures and a gradually decreasing percentage of tumors in the batches inoculated with materials which have been incubated at increasing temperatures are generally fully-grown tumors which have developed rapidly and come of a relatively active strain. They are usually softer and more cystic than those considered in the previous section. Their most marked characteristic is, however, a state of necrosis accompanied by an increasing calcium content without an entire disappearance of potassium.[10] This state of necrosis is peculiar in that it is apparently not attributable to the intervention of bacteria, very few of which are present in the most satisfactory transplantation materials, but rather to some auto-digestive or enzymotic process at work within the cells.[11]

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[10] Clowes and Frisbie, *American Journal of Physiology*, 1905, xiv, 173.

[11] In all those cases in which tumors have been infected they have proved totally unfit for transplantation experiments, leading either to the death of the mouse or to an ulceration process whereby the infected materials were entirely removed, no tumors resulting from such a treatment.

The enormous proliferative power possessed by the cells of this Jensen tumor has frequently been referred to by other workers who have employed the same materials. Such a remarkable development may most readily be explained as due to the intervention of some agent continually or intermittently stimulating the cells to further proliferation. We are not concerned at the present moment with the exact chemical composition of the body in question, as to whether it is possessed of an enzymotic or toxic nature or consists simply of a complex aggregate possessing a well-defined chemical composition. Neither are we attempting to determine the source of the body in question; whether it be derived from some parasite which has invaded the cell, or is itself a product of the impaired metabolism of the cancer cell. It is simply proposed to consider the probable action, at varying concentrations, of a chemical body capable of exerting a stimulating action on the cell which reaches an optimum at a given concentration and follow certain laws governing chemical action.

Poisons which cause the death of an organism at concentrations exceeding a given limit are in all cases capable of producing a stimulating effect upon that same organism when employed at sufficient dilution, whilst below that optimum point they produce little or no effect of any kind. Thus, for example, mercuric chloride inhibits absolutely the development of yeast at a concentration of one part in 20,000, whilst at a dilution of 1 in 330,000 the proliferative power of the yeast subjected to its action is actually increased.[12] The same effect is exerted upon enzymes; diastase, for example, is stimulated in its activity by the addition of extremely small amounts of sulphurous acid, oxalic acid, etc., whilst had the concentration been ten to forty times as great, the action of the diastase would have been nil, or practically inhibited.[13] It should be noted under this head that the stimulating effect of such toxic bodies is exerted only within extremely narrow limits, usually ranging from concentrations about five times as great to concentrations about five times as dilute as the optimum.

A second law which is applicable to the case in point is that governing the action of chemical bodies in solution at the same concentration but at varying temperatures, to the effect that with rising temperatures their activity is correspondingly increased. This law has been found to apply to bodies of the nature of enzymes, toxins, etc. Thus, for example, the activity of invertase in the conversion of cane-sugar into invert-sugar increases with increasing temperature until a point is reached at which physical conditions, such as precipitation of the enzyme, lead to the suspension of its function and to a decrease in activity.

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[12] Biernacki, *Pflüger's Archiv*, 1891, xlix, 112.

[13] See Duclaux, Traité de Microbiologie, vol. ii.

The recent work of Arrhenius and Madsen[14] on the hæmolysis of red blood corpuscles, making use of dilute solutions of toxins, such as diphtheria and tetanus, has demonstrated that these bodies exert a hydrolytic effect very similar to that of weak acids and bases and following the physico-chemical laws governing dilute solutions both as regards concentration and temperature variations.

If, as is highly probable, the proliferation of tumor cells is attributable to the presence of a toxic or chemical body acting as a stimulus, the concentration of the body in question most favorable to active proliferation probably lies within comparatively narrow limits, and consequently the slight changes in the concentration of this body would produce a marked variation in stimulus.

The second law considered above regarding the influence of temperature makes it appear probable that increased temperatures at a given concentration would produce a result similar to increase in concentration at the same temperature; in other words, an increase in stimulus to proliferation. Thus, where the concentration is too low to produce a marked effect at normal temperatures, an increased activity may be brought about by increased temperatures, the chemical energy of the stimulating body being thereby increased. On the other hand, the incubation of materials in which the stimulant was already present in the most favorable proportions for the normal temperatures would so increase the activity of the body in question as to exceed the optimum and eventually exert a destructive, inhibiting action upon the development of the cells themselves.

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[14] Festskrift af Indvielsen ved Statens Seruminstitut, Copenhagen, 1902.

That so short a treatment in saline solutions at temperatures equal to or not far in excess of the normal should produce so marked an effect may at first sight appear impossible. but the effect of the highly dissociated saline solution employed must not be ignored, and for rapidity of effect we have a perfect analogy in the results published by Calkins[15] on the proliferative energy of paramœcium. In certain of his series which had been maintained for considerable periods of time on a uniform diet and were consequently relatively inactive, the effect of subjecting them for a period of twenty-five to thirty minutes to the influence of such salts as potassium phosphate and potassium chloride, and once more returning them to the original medium was to increase greatly their rate of subdivision for a considerable period exceeding in time one or even two generations of our mouse tumors.

A careful consideration of the theory elaborated above may be made to throw much needed light on the perplexing problems which daily present themselves in studying cancer, more especially in connection with the transplantation of mouse tumors. Enzymes and toxins, like catalytic chemical agents, exhibit, as stated above, marked variations in activity with varying temperatures. Toxins and poisonous chemical bodies are capable at extreme dilution and within very narrow limits of concentration of exerting a marked stimulation upon the cell. We have ample evidence of the existence of a stimulating agent in the cancer cell. If this body is possessed of a chemical or enzymotic nature, it is only to be expected that incubation of the cells containing it, in dilute saline solutions, would lead to its liberation and transformation into a temporarily more active state. Such a stimulus, even applied for an extremely short time, is capable, as shown above, of exerting a prolonged effect on certain organisms. In our experiments the stimulating agent may be said to be present at optimum concentration for producing proliferation in those tumors which on direct inoculation produce yields of 80 per cent. and upwards. Consequently any increase in temperature may be expected to exert destructive action, the stimulating body thereby reaching a degree of activity which would lead rather to the destruction than to the stimulation of the cells. On the other hand, tumors possessed of a less marked proliferative energy, which have grown less rapidly, shown less increase, and yield a smaller percentage on direct inoculation, may be stimulated to produce an optimum effect at a temperature slightly above the normal; whilst those which under normal conditions give extremely small or practically no yield may be stimulated by even higher temperatures, the activity of the relatively small concentrations of the agent in question being so increased by incubation as to lead to a relatively increased yield after such a treatment.

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[15] *Journ. Exper. Zoöl.* 1904, i, 423.

That chemical stimuli exert an effect only within very narrow limits of concentration and only in the presence of suitable solvents may well account for the extraordinary difficulties sometimes experienced in obtaining successful and uniform transplantation of tumors. It may account for variation in the speed of development of individual tumors in the same series, for spontaneous recoveries, etc.

In conclusion, we may once more draw attention to the fact that those tumors which apparently contain excessive quantities of the body in question, are those which have recently reached maturity, have partially undergone necrosis, and contain relatively large proportions of calcium. The compounds of complex organic bodies with calcium are, for the most part, relatively insoluble as compared with those of potassium and sodium. It is conceivable that this extraordinarily rapid necrosis, without intervention of bacteria, of those tumors derived from the most virulent series associated with a preponderance of calcium over potassium,[16] is a reaction on the part of the organism whereby the active stimulating agent is at least for the time being removed from the scene of action by precipitation.

In such a case it is conceivable that the influence of incubation in a dilute saline solution is to convert the stimulating agent into an active, soluble form capable of exerting an effect upon any susceptible cells subjected to its action.

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[16] **Clowes and Frisbie,** *loc. cit.*

Such a stimulating agent as that referred to above is obviously not generated spontaneously, and it would be interesting to trace its possible source by observing to what extent the above results can be made to harmonize with the theories generally advanced regarding the origin of cancer. Owing, however, to the paucity of our present material it would be preferable to postpone the consideration of this question until we have more experimental data at our disposal.

A Study of the Influence Exerted by a Variety of Physical and Chemical Forces on the Virulence of Carcinoma in Mice, and of the Conditions Under Which Immunity Against Cancer May be Experimentally Induced in These Animals.*

***Being a Paper read in the Section of Pathology at the Annual Meeting of the British Medical Association, Toronto, August,* 1906.**

By G. H. A. Clowes, Ph.D.

The discovery of the ease with which mouse tumors may be transplanted from one individual to another has led to the development in the course of the last few years of an entirely new field of experimental research in cancer. A large amount of data regarding the nature of these mouse tumors and the conditions under which immunity may be established against them has been accumulated in the New York State Cancer Laboratory in the last two years. This work has been carried out for the most part by Dr. Gaylord and the writer, with the co-operation of Professor Calkins of Columbia University, and the assistance of Mr. Baeslack and other members of the laboratory staff.

It is proposed to give a brief summary of certain biophysical and biochemical experiments dealing with the stimulating or inhibiting action of heat, and of certain chemical bodies upon tumor materials. It is also proposed to present evidence regarding the existence of a definite immunity against tumor development, and to indicate briefly the methods whereby an artificial immunity has been induced in certain cases.

In addition to the Jensen tumor, for the gift of which we are indebted to Professor Jensen of Copenhagen, 35 primary tumors have been placed at the disposal of the laboratory in the course of the last two years. Of 15 employed for transplantation 3 which were infected caused the death of the mice employed, and 7 out of the remaining 12 gave positive results, 18 tumors being obtained from 204 mice inoculated.

It will thus be seen that our percentage yield from primary tumors compares with that of Bashford and is higher than that of Ehrlich.

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Three distinct strains of mouse carcinoma derived from entirely independent sources are now being employed in our laboratory for experimental purposes.

1. The well-known Jensen tumor, the virulence of which, whilst varying within very considerable limits, shows an average over any great length of time of about 30 per cent., both in our hands and in those of other investigators. This tumor develops relatively slowly, causing the death of the mouse in the average of a large number of cases in fifty-six days from the date of inoculation. About 22 per cent. of the tumors of this series recover spontaneously.

2. Known as the Brooklyn tumor, derived from a primary tumor sent to the Gratwick Laboratory in May, 1905. This tumor has now reached, in fifteen generations of transplantation, an exceptionally high degree of virulence, the last 612 mice inoculated having given 557 fatal tumors, or 91 per cent. The average duration of the life of the mice from the date of inoculation to the date of death has been twenty-six days, the average weight of the tumors slightly over 4 grams at death. This tumor was fairly virulent at the start, giving 5 tumors in 28 mice in the first generation, and it is interesting to note that with transplantation through succeeding generations the percentage yield of tumors rapidly increased, whilst the period in days intervening between the date of inoculation and the fatal termination of the disease steadily diminished. Thus the average of the first, second, and third generations shows 19 per cent. of tumors requiring an average of seventy-four days for development. The fourth generation shows 46 per cent. of tumors requiring forty-one days for development. The fifth and sixth generations show 69 per cent. of tumors, with an average of thirty-two days; whilst, as stated above, the seventh to the fifteenth generations have shown over 90 per cent. of tumors, requiring an average of twenty-five to twenty-six days for development. The great increase in virulence in this series is certainly not attributable to rapid transplantation of small tumors, as seems to have been the case in Ehrlich's highly virulent series. We find, on the contrary, that tumors under 2 grams give very unsatisfactory results on transplantation, whilst the best yields are obtained from large, moder-

ately-necrotic tumors which have already reached a stage at which the animal may be expected to succumb in the course of two or three days. The number of spontaneous recoveries occurring in this series is extremely small, at the present time less than 4 per cent.

3. A tumor known as the Springfield tumor, possessed of a very low degree of virulence, far less than that of the Brooklyn, and considerably less than that of the Jensen tumors. This series has only been established and maintained by incubation of the tumor materials at temperatures ranging between 38.5° and 41° C. for half an hour or more previous to inoculation. Primary tumors of this type had invariably failed to produce tumors in the following generation until the incubation method was employed, 13 tumors being obtained from incubated material before any were obtained from normal material. A comparison of the normal and incubated lots in this series shows at the present time 20 per cent. of tumors in the normal as against 57½ per cent. in the incubated. The relatively low virulence of this tumor at the start was associated, as might be expected, with a fairly high percentage of spontaneous recoveries, over 30 per cent. The series being now only in the seventh generation, it is too early to make any positive statements regarding its exact degree of virulence and tendency to retrogression.

From the statistical results recorded above it must be concluded that:

1. The virulence of a tumor bears a definite relationship to the average speed of development of the tumor; that is to say, the larger the percentage yield obtained on transplantation of a tumor the more rapid will be the development of the tumors of that series and *vice versa.*

2. The greater the virulence or speed of development of a given series of tumors the smaller will be the proportion of spontaneous recoveries in that series, and *vice versa.*

EXPERIMENTAL METHODS

Every possible effort has been made to eliminate so far as possible those errors commonly associated with animal experiments, both by employing large numbers and also by insuring a uniform dosage on inoculation. The procedure is as follows:

A tumor is ground up in a mortar with a given volume of salt solution, the connective tissue residues removed by means of fine rakes, and uniform doses of the macerated tumor material introduced subcutaneously into each mouse of a series. In case it is proposed to study the relative effect of a series of chemicals or any other agent like blood serum, calculated to inhibit the development of the tumor cells, equal amounts of the uniform macerated cancer material are treated with uniform doses of the bodies in question, and allowed to stand for equal periods of time. Mice derived from the same source and of the same size are then divided into a series of lots containing ten or more individuals apiece. A batch of mice is employed for each of the prepared admixtures of cancer and chemical or serum, the greatest care being observed that not only do all mice in the same batch receive an exactly equal dose, but also that all mice in the same series receive exactly the same amount of cancer material, so that the only varying factor is the chemical body or serum admixed therewith.

Further, in order to maintain a complete control of every individual mouse in each series, with a view to observing the occurrence of tumors, their speed of development, and final fatal termination or spontaneous recovery, a system of charting has been introduced whereby a permanent record of the progress of each tumor from the date of inoculation to the date of death of the animal is preserved in the laboratory.

SPONTANEOUS RECOVERIES [1]

The adoption of the above charting system enabled us to detect the occurrence of spontaneous recoveries very shortly after the commencement of the transplantation work on the Jensen tumors. Excluding all tumors the diameter of which was not equal to 3½ mm. not less than twenty days after inoculation, we found that upwards of 100 definite spontaneous recoveries had occurred in the Jensen series alone previous to January 1st of this year, the tumors varying in size from the minimum to a weight of three or four grams.

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[1] For references see page 109.

To summarize the statistical results obtained in this work, it may be said that the larger the yield of tumors and the more rapid their growth the smaller is the number of spontaneous recoveries; and also that the more advanced is the tumor in its development the smaller are its chances of retrograding. It would in fact, appear probable that a very large number of spontaneous recoveries occur previous to the time at which the tumor has reached such dimensions as to be readily recognizable.

INFLUENCE OF INCUBATION [2]

Whilst all tumor cells appear to be destroyed at 45° C., incubation of the macerated tumor materials previous to injection, at temperatures ranging from 38.5° C. to 41° C., for periods of twenty minutes to half an hour, appears to exert a stimulating effect upon tumors possessed of a low degree of virulence. On the other hand, tumors possessed of a high degree of virulence suffer considerable attenuation when subjected to such a treatment. This remarkable fact was first recognized whilst attempting to immunize animals against subsequent injections of tumor materials by treating them with incubated material, which it was anticipated would lose thereby in virulence to a sufficient extent to enable the animal to overcome it by natural means. The incubation of weak tumors, as stated above, has proved of considerable value from an experimental standpoint, one series of tumors having been established entirely in this manner.

ACTION OF CHEMICALS IN INHIBITING DEVELOPMENT OF TUMORS

In a considerable series of experiments mercuric chloride, potassium cyanide, ammonium fluoride, and other bodies of this nature were admixed with normal tumor materials in varying proportions with a view to determining —

1. The point at which development of the tumor cells would be absolutely inhibited.
2. The action which materials treated in this manner would have in developing an immunity in the animals so treated.
3. A possible means of removing bacteria from infected tumors.

The resistance of the tumor cells to inorganic disinfectants was found to be extremely high. Treatment of the cancer material for one hour previous to inoculation with mercuric chloride at a concentration of 1 in 3,500 was insufficient to prevent the development of a small proportion of slow-growing tumors, final complete destruction of the cells being effected first at a concentration of 1 in 2,000.

Mercuric iodide effected a similar result at a concentration of from 1 in 2,000 to 1 in 2,500.

The action of potassium cyanide was most remarkable. The powerful, highly-virulent Brooklyn tumor still gave 100 per cent. yield after treatment with potassium cyanide at a concentration of N/250, and tumors were produced both in this and the Jensen series when the concentration of potassium cyanide was raised to N/100. In one experiment in which ten mice were employed, the concentration of potassium cyanide was so high that half the mice succumbed within a couple of hours after receiving the injection, in spite of which one large and one small tumor developed in the remaining five, thus showing that the resistance of a tumor cell to potassium cyanide is greater than that of the mouse itself.

Bacteria are entirely destroyed by treatment with potassium cyanide at a strength of N/200, and in one series in which the tumors were seriously infected, thus causing the death of the animals, the treatment of a portion of a tumor with potassium cyanide at a concentration of N/200 resulted in saving a considerable portion of the animals and the production of several tumors.

Ammonium fluoride inhibits all tumor development at a concentration of 1 in 1,000, a few small tumors making their appearance at 1 in 2,000.

The reinoculation of those mice which received the injections of materials which had been previously rendered innocuous through treatment with chemicals gave no indications of immunity.

Careful examination of the percentages of tumors in the varying groups, and the rate at which the individual tumors develop, would indicate that not only do the tumor cells offer a great resistance to the action of the disinfectants referred to above, but also that at suitable concentration a distinct stimulating effect may be observed when compared with the normal, similar to that observed by Biernecke in studying yeasts.

The discovery that rapidly-growing tumors contain a large proportion of potassium[3] and slow-growing tumors a large proportion of calcium, led us to test the effect of substituting isotonic solutions of potassium and calcium chloride for the ordinary salt solution in admixture with the cancer material. The mice which received potassium injections and were subsequently fed on food containing potassium in considerable quantities showed a larger percentage of tumors than did those treated in a similar manner with calcium. We propose, however, to repeat this experiment before attaching any importance to the results obtained.

IMMUNITY

It is necessary at this stage to refer briefly to the results already published on immunity against cancer. That immune forces exist antagonistic to cancer development in mice is now an established fact. In January, 1905, a paper was published from our laboratory and read at Johns Hopkins University,[4] in which it was stated:

1. That in the Jensen series mice recover from true tumors in at least 20 per cent. of cases.
2. That small tumors treated with serum of such mice might be retarded in development and in a few cases cured, whilst those treated with normal serum showed a less marked effect.

Whilst a considerable number of such recoveries had already been reinoculated, it was not possible at that early stage to furnish any reliable data regarding the chances of their developing tumors. On July 15, 1905, a second paper,[5] affording further evidence of immunity, was sent to the publisher. In this paper it was shown that thirty spontaneous recoveries from genuine tumors on reinoculation, many of them for the third time, had failed to show a single tumor, whilst those which had not recovered from tumors on first inoculation showed a few tumors, and normal animals the usual 30 per cent. yield. In one series the mice were intentionally inoculated with an extremely weak strain, with the result that 50 per cent. showed abortive tumors, and subsequently recovered, whilst the remainder gave no indication of tumor development. Those that had recovered on the first occasion failed entirely to develop tumors on further inoculation with a far more virulent material, whilst the remainder that had not developed tumors on the first inoculation showed a fair proportion of tumors after inoculation with the more vigorous strain.

A further experiment carried out at this time, treating tumor materials previous to inoculation with the serum of spontaneously recovered mice, in comparison with that of normal mice, showed that whilst 31 per cent. of tumors were obtained in the normal series, only 12 per cent. were obtained in the immune series, or, if rapidly-growing tumors only were considered, 24.8 per cent. in the normal series, as against 5.6 per cent. in the immune series. In his report[6] of that year Bashford criticized our preliminary communication, stating:

1. That only one spontaneous recovery had occurred in his experience with 3,000 tumors in the Jensen series after fourteen days' growth, and that our large percentage of spontaneous recoveries was to be accounted for by assuming that some septic process following inoculation had given rise to swellings in the subcutaneous tissue which were in no sense to be looked upon as tumors.

2. That whilst transplantation fails on the first attempt in a certain number of animals, subsequent inoculation gives almost the same percentage as first obtained, and that the variations in the success attending transplantation in unused mice, and any mice which have been inoculated unsuccessfully, are not greater than those obtaining in different series of unused mice.

Early this year, a paper dealing with spontaneous cure of cancer[1] was published from this laboratory, in which upwards of 100 such spontaneous cures were carefully investigated from a pathological and statistical standpoint, and no doubt remains that true, growing tumors had existed in every one of the cases dealt with. Subsequent efforts to reinoculate these 100 mice were without result. Bashford's contentions that our spontaneous recoveries were ulcerations and that all mice might be reinoculated successfully, were, therefore, both disposed of.

Unfortunately, owing to a severe epidemic in which we lost upwards of 3,000 animals and barely retained our valuable tumor mice for subsequent work, a large proportion of the spontaneous recoveries were lost in the course of the winter, and it has consequently been found impossible, up to the present time, to repeat the experiments referred to above, treating tumor material with immune serum previous to injection.

In April of this year, Ehrlich[7] published an exhaustive discussion regarding immunity against mouse tumors, in which he demonstrated, with figures more elaborate than those which we, with our smaller facilities, were able to present, that mice which have survived a first inoculation, even with attenuated materials, may remain immune when reinoculated for a second and third time with a far more virulent strain than that originally employed, a smaller percentage of tumors being obtained on each occasion, until finally the remaining mice are immune to all further treatment.

Ehrlich states (p. 89) that we had recognized the existence of immune forces in mice that had recovered spontaneously from cancer. He further notes, in a subsequent sentence, that Michaelis, after injecting carcinoma material killed by means of chloroform, had failed to find an immunity in mice so treated, an observation which he apparently considers contradictory to our results. Ehrlich then states on page 90 that, "instead of using chemically-destroyed carcinomatous material, as had his predecessors in this line of research," he was himself employing a living but weakened tumor strain as a means of inducing immunity. Ehrlich appears to have accidentally overlooked the fact that we were using mice which had recovered spontaneously from growing tumors, and have included our work with that of others who had attempted unsuccessfully to immunize mice by means of inanimate materials. Our very first experiences, more than two years ago, demonstrated the futility of any such method as that which Michaelis had employed, and we were the first to emphasize the role played by the living cell in inducing immunity. We still believe that an actual growth must take place in order that immunity may be conferred. We are, and since the commencement of our experiments have always been, convinced that immunity against cancer is not induced by means of any inanimate material, but only by the intervention of the living cells or virus.

A paper has just been published by Bashford[8] in which he apparently repudiates his own statements of the previous year, and confirms our results, although no statement appears to that effect. Bashford recognizes the occurrence of spontaneous recoveries in large numbers (in certain series as many as 50 per cent.), and, further, that on reinoculation a very small percentage of tumors is obtained from such recovered mice as compared with normal controls — a corroboration of our results. He finds, further, that all mice are not reinoculable, as stated in his previous report, and, whilst he obtains nearly the same percentage of tumors on the second as on the first inoculation, eventually a stage is reached at which the percentage yield on reinoculation is much lower than that of normal control animals — a further confirmation of the results obtained by Ehrlich and ourselves.

He states that body fluids of some protected mice when injected into mice with experimental cancer have given indications of a power to retard the growth of well-established tumors — a confirmation of the statement in our first preliminary report, to the effect that serum of spontaneously recovered mice exerts a more powerful influence on tumors in other mice than does the serum of normal individuals, leading in certain cases to a recovery and in others to a distinct retardation in the development of the tumor.

By making use of a feeble primary tumor, in which a large majority of the tumors that appeared recovered spontaneously, and subsequent inoculation with a more virulent strain, Bashford was able to demonstrate that a smaller proportion of tumors develop in those that so recovered, than in normal control animals, a confirmation of similar results obtained on a larger scale by Professor Ehrlich.

Finally, we would take exception to Bashford's statement that "we have at our disposal a means of protecting healthy mice from all the consequences of inoculating them with experimental cancer." Such a statement is, to say the least, misleading, unless, indeed, the loss of a certain number of individuals in the process of immunization is ignored. It is to be hoped that in the future means will be discovered of immunizing mice with nonliving material. So long as it is necessary to take the risk of losing a certain percentage when inoculating with attenuated materials in order to confer immunity against more virulent materials, it cannot be stated that we have a means at our disposal of protecting healthy mice at pleasure.

Since the completion of the work referred to above we have carried out further experiments in the New York State Laboratory directed toward the demonstration of the existence of immunity. I would call your attention especially to one experiment demonstrating the existence of immune forces distributed throughout the bodies of animals already developing tumors of considerable size.

Very early in our experiments, the careful observation of charted records led us to recognize the fact that a large number of tumors apparently remain stationary at a certain stage of development, and at that time are more susceptible to the influence of X-rays, serum, and various chemical agents. Recognizing this fact, so soon as we had tumors of great virulence at our disposal, in which a yield of 95 per cent. could be relied upon, we started the following series of experiments, which, whilst carried out independently of Professor Ehrlich, must be looked upon as a confirmation of the results included in his recent paper under the heading of " Double Inoculations."

Ten series each of ten mice were inoculated at the head with a virulent tumor, with the result that the large majority showed developing tumors of a fair size in the course of five or six days. All those that showed such tumors were inoculated at periods from five to ten days after the first inoculation with a second dose of a tumor of the same or even more virulent strain, at the tail; in each case a series of unused animals being inoculated with the same materials at the same point, as a control. Whilst in the control series in the course of twelve or fourteen days from the time of inoculation, a yield of 95 per cent. of large tumors was obtained, in the series which had already had tumors at the head, previous to the second inoculation at the tail, only one or two small tumors were to be found at the tail, the yield being less than 6 per cent. The fact that the presence of a fair-sized growing tumor inhibits the development of materials of the same virulence, and even of materials of greater virulence at the second point of inoculation, indicates to our minds the probability of the existence of powerful immune bodies in the serum.

It has long been recognized that metastases are formed with difficulty, and only in a relatively small percentage of cases reach any considerable size even in those mice which have large tumors.

It may well be supposed, from the above experiments, that the growth of a tumor meets with an energetic resistance on the part of the host; that antibodies of some type antagonistic to the development of tumor cells are produced in the serum, which, whilst powerful enough to inhibit the development of isolated cells, such as would occur in secondary inoculations and metastasis formation, are not necessarily sufficiently powerful to inhibit the development of the more virulent tumors. When, however, a tumor is retarded in its development, remains stationary, and eventually recovers spontaneously, it may well be supposed that such recovery is due to the natural production in the body fluids of the animal of antibodies antagonistic either to the tumor cells in their development or to some body within those cells stimulating them to proliferation. This theory finds support in the fact that such spontaneous recoveries are not reinoculable with tumors of the same virulence and show less tendency to inoculation with tumors of a higher grade of virulence than do normal mice; the resistance being apparently larger, the larger the tumor from which the mouse originally recovered.

We have collected the data accumulated in this laboratory regarding the reinoculation of mice which had failed to give tumors on the first inoculation. Excluding those cases in which the use of chemicals and high temperatures may be expected to have destroyed the tumor materials employed, it may be said that in the Jensen series from 30 to 35 per cent. of tumors are obtained on first inoculation; the second inoculation giving 10 per cent., and the third *nil*. We regret that the occurrence of an epidemic rendered it impossible to inoculate these mice, as would otherwise have been done, a considerable number of times.

In the Brooklyn series the last 400 mice have yielded 95 per cent. of tumors; the remaining 20, 3 or 4 of which had recovered spontaneously, gave 3 tumors on reinoculation. The immunity developed is not, therefore, as great as that observed by Ehrlich under similar conditions. The reinoculation of the survivors of less virulent strains, such as the Jensen and Springfield strain, with our more virulent tumor, whilst giving a yield considerably below that normally obtained from unused mice, would not indicate, especially if large doses are employed, that as great a degree of immunity had been conferred, as was found by Ehrlich in the results reported in his last communication on this subject. This difference may, however, be accounted for by the difference in our methods of transplantation, the probability being that a larger number of free tumor cells are introduced by the method which we employ than by that used in Ehrlich's laboratory; in which case the effect exerted by immune forces might be expected to be relatively less.

The evidences of immunity so far presented are:

1. The occurrence of spontaneous recovery from true tumors.
2. Such spontaneous recoveries are not reinoculable.
3. The reinoculation of mice which have failed to develop tumors on the first occasion with a tumor of an equal virulence leads to a reduction in the percentage, in Jensen tumor from 35 to 10 per cent., and in the virulent Brooklyn tumor from 95 per cent. to 20 per cent.
4. The reinoculation with virulent materials of mice previously inoculated with a weaker strain leads, as has been found by Ehrlich, to a reduction in the percentage of tumors as compared with the normal.
5. When mice are suffering from tumors of a certain size it is not possible, as found by Ehrlich and ourselves, to bring about development of a tumor at a second point by further inoculation.
6. The serum of recovered animals apparently produces a certain effect upon small tumors in other animals.
7. The serum of recovered mice appears to exert an inhibiting or retarding effect upon the development of macerated tumor material when admixed with the latter previous to inoculation.

ATTEMPTS AT ARTIFICIAL IMMUNIZATION BY MEANS OF INANIMATE MATERIAL

Tumor materials were incubated at a temperature sufficiently high to entirely destroy the cells, so that no tumor development took place on subsequent inoculation. The mice, on reinoculation, after a considerable period showed no evidences of immunity.

The treatment of tumor materials previous to inoculation with chemicals, such as mercuric chloride, potassium cyanide, iodine, etc., at a concentration just sufficient to prevent tumor development, and subsequent inoculation of mice with this material, failed to confer on these animals any degree of immunity as compared with the normal.

The treatment of tumor materials previous to inoculation with immune serums — that is to say, the serums of mice which have recovered from cancer, whilst reducing the percentage of tumors on that occasion, does not confer upon the mice an immunity against further inoculation, these groups showing if anything a larger yield of tumors on the second inoculation than do other reinoculated series.

Chemical analyses[3] of over 350 tumors have shown that nucleoproteids, potassium, and other constituents of the cell nuclei were present in a relatively large amount in tumors possessed of a high degree of virulence which had developed rapidly, whilst the reverse was the case in slow-growing, feeble tumors, in which the element calcium was found to preponderate. This being the case, it was decided to attempt to immunize mice by means of one of the following processes, following the lines adopted by Beebe in his work on exophthalmic goitre: A suitable weight of tumor material was extracted with water or weak alkali for a couple of hours, filtered, and precipitated with acetic acid. The centrifuged precipitate was washed and redissolved in weak alkali, filtered, subsequently precipitated, and once more redissolved and used in definite doses in a series of mice, rats, rabbits, and chickens. The animals employed received four or five doses, increasing in amount, at intervals of from five days to a week, the amount finally injected being from eight to twelve times that used on the first occasion — a dose which would have proved fatal for many mice. The tumors of upwards of 300 mice, belonging for the most part to our virulent strains, were used for this experiment, and an attempt was made to immunize in all 250 mice. The animals were inoculated from a week to fourteen days after receiving the last dose, but we have not so far been able to observe any indications of an immunity having been conferred upon the mice by such a direct treatment. The rats, rabbits, and chickens which had also received increasing doses of nucleo-proteids were killed and their serum used in comparison with that of normal animals of the same species, employing the method of admixing macerated cancer materials with the serums for an hour previous to injection. No result was obtained from rat and rabbit serum. In one case the serum of an immunized chicken appeared to retard development to a very slight extent; but since three other chickens similarly treated gave no result, this result will require further confirmation.

The nucleo-histons present in tumors were isolated by extracting with weak salt solution, precipitating with calcium chloride, centrifuging, dissolving in 3 per cent, sodium chloride, dialyzing, reprecipitating, etc. These nucleo-histons were used in the same manner as the nucleo-proteids, increasing doses being employed at intervals of five or six days. The results were in every sense negative.

From the above experiments it will be seen that great difficulties are to be anticipated in any attempt to immunize animals by means of dead material.

THEORETICAL CONSIDERATIONS

In conclusion it is necessary to refer briefly to certain interesting and suggestive theories recently advanced by Professor Ehrlich, by means of which he attempts to explain the proliferative energy of tumor cells, and the indications of immunity referred to above. Adopting his side-chain theory as a working basis, and assuming that all cells are possessed of side-chains or receptors, by means of which the necessary foodstuffs are attached to the cell, he considers the abnormally rapid growth of tumor cells is attributable either to an increased number of such receptors or to an increase in the affinity possessed by such receptors for foodstuffs. As a result, in the establishment of equilibrium in the system, the cancer cells obtain a larger proportion of the foodstuffs available than do the other cells of the body. As a result of his experiments, Ehrlich concludes that the normal food affinities of the cell are either stimulated by a specific X body, the nature of which he does not further describe, or that some peculiar nutritive material essential to the development of the cancer cell has to be supplied, and in its absence the cancer cell fails to develop. He is, however, forced, in certain cases, to the conclusion that the immune forces exhibited, both by rats and mice that have developed an immunity after one or two injections of tumor material, are attributable to specific antibodies induced in the serum which he looks upon as cytolysins, and the formation of which he explains by assuming that the receptors of the tumor cells have become sufficiently changed to permit of the production of antibodies to them, which do not affect those of the normal body cells.

In developing a theory conforming with Ribbert's existing theory regarding the origin of cancer, Ehrlich has apparently ignored the possibility of some active stimulating agent foreign to the body being the cause of cell proliferation. It is well known that the normal protoplasmic functions can be stimulated to increased activity by chemicals, poisons, and bacterial toxins, and it appears to us possible to explain the experimental results referred to above by assuming that the stimulus to proliferation both of the original cell and its descendants may be supplied by the secretion of some virus within the cell, or immediately in its neighborhood. Such stimulus would, in all probability, lead to cell division associated with an increased capacity for food absorption along the lines depicted by Ehrlich, but rather as effect than cause. Our experiments would indicate that immunity is conferred as a result of active growth followed by recovery, some antibody being produced in the serum antagonistic either to the cell development, or more probably to that of the agent stimulating the growth of the cancer cell.

Evidence in support of the theory that some virus acting as a stimulating agent either on epithelial or connective tissue cells leads indirectly to the formation of carcinoma or sarcoma, and that immunity against cancer is the result of a reaction against such a virus on the part of the body, is afforded by the following observations:

1. Ehrlich finds that a mouse which, through inoculation, has become immune to carcinoma is also immune to sarcoma and vice versa, thus indicating a reaction against some common agent. Ehrlich assumes the existence of a specific *Wuchsstoff* or x body, which, when attached to an epithelial cell, gives it the character of carcinoma, and when attached to a connective cell, that of sarcoma, and he evidently looks upon this body as an agent capable of stimulating the ordinary receptors of the cell in question, giving them an increased affinity for the assimilation of foodstuffs; but might not the stimulus in question be equally well attributed to the action of some common virus capable of infecting cells of either type?

2. Hemorrhage is apparently very frequently associated with retrogression of tumors. Any treatment tending to bring about hemorrhage retards the development of tumors, and as Ehrlich has pointed out, those tumors which were originally hemorrhagic, show the least tendency to produce tumors when transplanted, indicating that the presence of blood serum is anything but favorable to tumor development, attributable in all probability to the presence of antibodies.

3. The serums of animals which have recovered from tumors exert an inhibiting effect both on tumors in other animals and also on macerated cancer material previous to inoculation — a fact which can scarcely be explained by other means than the assumption of antibodies directly antagonistic to the development of the cells, and since the test tube experiments carried out in this laboratory would indicate that the serum in question is not possessed of any marked hemolytic or cytolytic characteristics, it would appear probable that the effect is exerted directly upon some virus within the cell, and not upon the cell itself.

4. The following observations made both by Ehrlich and ourselves:

A. That large metastases occur very seldom until near the close of the animal's life.

B. That if mice already developing tumors are inoculated for a second time from five to ten days after the first inoculation, even with a more virulent material than on the first occasion, tumors are practically never produced, and, even if produced, develop far more slowly than in normal control animals.

If, as Ehrlich believes, virulence is simply dependent upon the relative ability of the cell receptors to attach foodstuffs, the establishment of equilibrium should certainly permit of the development of the second tumor, since the receptors of the latter are supposed to be more active than are those of the normal body cells, and either equal to or more virulent than are those of the first tumor. Ehrlich explains this phenomenon by assuming that the original tumor, being well established and having a good blood supply, grasps all available foodstuffs for itself, thus depriving the cells injected on the second occasion of the necessary nourishment. Since, however, the whole question, according to Ehrlich, resolves itself into one of the establishment of chemical equilibrium between the active food receptors of a series of cells and the available supply of foodstuffs, the cells of the second tumor should at least be able to overcome the weak affinities of the normal body cells, and there is every reason to anticipate that they should develop; but, as stated above, this is practically never the case, and at autopsy merely the smallest residue of connective tissue is usually to be found at the point of inoculation. It is, however, possible that immune forces are induced in the serum, as a result of reaction against the developing tumor, powerful enough to affect the virus within the isolated cells introduced on the second occasion, but not sufficiently powerful to penetrate and destroy completely the original well-established tumor.

Further support of this point of view is found in the fact which we have frequently observed, that considerable quantities of tumor material must be employed on inoculation if the maximum yield is to be obtained, indicating the existence of immune bodies even in the serum of normal mice, which, when distributed over a large number of cells, are incapable of producing as great an effect as would be the case were the smaller amount of tumor material employed.

That immune forces are produced in the serum in gradually increasing quantities as the tumor progresses, is indicated by the tendency frequently exhibited by tumors to remain stationary after fairly rapid development, and then subsequently either to grow rapidly once more or to retrogress. In this connection it must be noted that X-rays produce a much more marked effect at this stage than at a subsequent stage, at which apparently the tumor has quite overcome the natural immunity of the mouse and is developing rapidly.

5. Perhaps the best evidence in favor of the theory of a stimulating virus is afforded by the following experiment published from this laboratory, July, 1905, as follows:

> A group of upwards of 50 mice were inoculated with an exceptionally weak strain of the Jensen tumor, with the result that only 1 fatal tumor was produced. A large number of abortive tumors resulted, and in one particular group, 209, in which 3 mice showed no tumor development and 3 recovered spontaneously, a subsequent inoculation with a more virulent Jensen strain gave no tumors in those which had previously recovered spontaneously from the weaker strain, whereas in the other 3 tumors made their appearance, 1 of them subsequently proving fatal. If it were a question of affinity for foodstuffs with which we had to deal, why did not tumors develop in all of the mice on the first occasion, or in those that had spontaneously recovered on reinoculation? It would appear more probable that the 3 mice that failed to show tumors on the first occasion were possessed of a weak immunity, insufficient, however, to protect them against the stronger materials on the second inoculation, whereas those 3 which developed the disease on the first occasion, and subsequently spontaneously recovered, possessed as a result an immunity which was sufficiently strong to protect them against all subsequent inoculations, even with vastly more virulent materials.

6. The great variation in the speed of development of tumors in the same series, the variation in the speed of development of the same tumor at different stages, the almost complete recovery, and subsequent fatal termination of certain tumors, and especially the influence of incubation on weak tumors, stimulating thereby to more active proliferation, appear to conform with the conception of a stimulating virus.

Ehrlich has presented one series of experiments which is certainly capable of a more ready explanation by his assumption of a specific *Wuchsstoff*. He found that a virulent mouse tumor was capable of growing for a short time in a rat, but subsequently retrograded. It was further demonstrated that such a tumor after remaining for four or five days in a rat, could still be transplanted to a mouse but not to a normal rat, neither could the rat which had recovered be further inoculated. Ehrlich explains this by assuming that the mouse tumor introduced into the rat carries with it a certain amount of a specific *Wuchsstoff;* that it is capable of assimilating ordinary foodstuffs from the rat, but that so soon as its specific *Wuchsstoff* is exhausted it succumbs. Transplantation back to a mouse provides the cells once more with their specific *Wuchsstoff,* and enables them to develop, so that they are once more capable of transplantation in a rat for a short period of time; on the other hand, when passed from the first rat into a second rat, the absence of the *Wuchsstoff* in the rat prevents further development. He argues that since the mouse tumor is apparently still possessed of the same virulence after spending a certain time in the rat, the failure to take in the second rat is not attributable to attenuation, as would appear to be the case. It is, however, perfectly conceivable that the virus capable of stimulating mouse cells to proliferation is incapable of living in a rat — at any rate, for any length of time.

Ehrlich recognizes the existence of antibodies conferring immunity on those mice which, after the first or second inoculation with attenuated materials, prove incapable of developing tumors on subsequent inoculation.

The experiments enumerated above, when considered in conjunction with the cases of cage infection reported by Gaylord from this laboratory, offer very strong evidence in support of the theory that the cell is stimulated to proliferate by a parasite, or the toxins produced by a parasite which has infected the cell. If such a parasitic stimulus may be considered identical with Ehrlich's specific x body or *Wuchsstoff,* a ready explanation of the subsequent process of proliferation may be derived from Ehrlich's theory. The close correspondence in the experimental results obtained by Ehrlich and ourselves is of considerable interest in view of the fact that the materials employed were derived for the most part from entirely independent sources, and the experiments carried out under widely differing conditions. The existence of a definite immunity against cancer in mice has been demonstrated in a variety of ways, and any slight difference of opinion regarding the theoretical interpretation of certain experimental results should only lead to renewed efforts directed toward the final solution of the problem.

SUMMARY AND CONCLUSIONS

From the above experiments, in which over 7,000 mice have been employed, it may be concluded that:—

1. Primary tumors are only transplanted with great difficulty; after the first generation the yield of tumors gradually increases until a maximum virulence is attained, which subsequently remains fairly constant for a considerable period of time.

2. Increase in virulence of a tumor strain is invariably associated with an increased rate of growth of the individual tumors.

3. The proportion of tumor mice recovering spontaneously in any series is apparently inversely proportional to the virulence and speed of development of the tumors of that series.

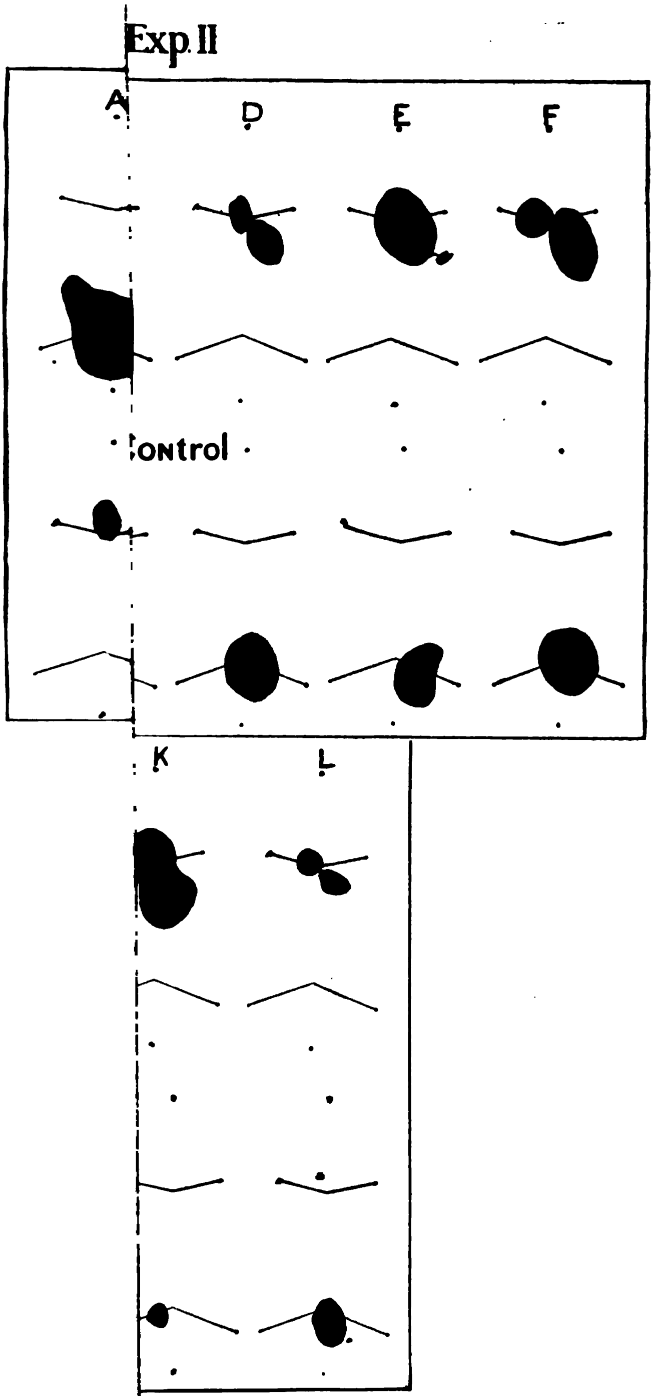
4. The larger the dimensions actually reached by a tumor the smaller are the chances that it will recover spontaneously.

5. Incubation of tumors possessed of a low grade of virulence, previous to injection into mice, is found to exert a stimulating effect, larger yields of tumors being obtained than in control series.

6. The resistance of tumor cells to mercuric chloride and other inorganic disinfectants is very high. It was found possible, for example, to destroy the bacteria present in badly-infected tumors by means of potassium cyanide without seriously affecting the virulence of the tumor on subsequent transplantation.

7. The chemical analysis of over 300 tumors shows a relatively high potassium and nucleo-proteid content, associated with high virulence and rapid development, and a low potassium and high calcium content, associated with low virulence and relatively slow development.

8. The principal evidence of the existence of immunity against cancer is as follows: Spontaneous recovery of mice from true tumors actually occurs. Those mice which have recovered are not reinoculable with tumor materials possessed of the same degree of virulence as that previously employed, and exhibit in



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addition a considerable immunity to subsequent injections of far more virulent strains. The reinoculation of mice which have failed to develop fatal tumors shows in our experience a great reduction in the proportion of tumors, and inoculation for a third time has so far failed to be productive of a single tumor. The serum of recovered mice apparently exerts a definite though slight effect on the small tumors in other mice when directly injected, and also on tumor materials when admixed previous to inoculation. Mice on which tumors are already developing are, with a few exceptions, immune to subsequent inoculation, even with a more virulent tumor, indicating the production of immune forces in the serum antagonistic to the development of cancer.

9. The injection of tumor materials incubated at such temperatures as to render development impossible, or of tumor materials previously treated with chemicals at such a concentration as to inhibit development, fails entirely to confer immunity on the mice so treated.

10. The treatment of mice with increasing doses of nucleoproteids (extracted from the most virulent tumors) at stated intervals of time has so far failed to confer an immunity.

11. The process of immunizing mice against cancer appears to be analogous to that of vaccination against small-pox, the animals which recover from the attenuated form of the disease developing an immunity capable of protecting them in the large majority of cases against injections of a more virulent cancer strain than that originally employed.

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[1] Gaylord and Clowes, On Spontaneous Cure of Cancer, *Surgery, Gynaecology, and Obstetrics*, vol. ii, p. 633. [2] Clowes and Baeslack, Incubation of Mouse Tumors, *Journ. of Exper. Med.*, August, 1906. [3] Clowes and Frisbie, Potassium and Calcium Contents of Mouse Tumors, etc., *Amer. Journ. of Physiology*, 1905. [4] *Johns Hopkins Bulletin*, vol. xvi, No. 169. [5] Clowes and Baeslack, Further Evidences of Immunity against Cancer, *Med. News*, 1905. [6] E. F. Bashford, Report of Imperial Cancer Research Fund, 1905. [7] Ehrlich, *Experimentelle Karzinomstudien*, 1906. [8] Bashford, *British Medical Journal*, July, 1906.

Evidence that Infected Cages are the Source of Spontaneous Cancer Developing Among Small Caged Animals.*

HARVEY R. GAYLORD, M.D., and G. H. A. CLOWES, Ph.D.

The employment of small animals suffering from cancer for purposes of laboratory experimentation has brought to light the fact that the endemic occurrence of cancer in animals is a not uncommon phenomenon. The classical case of carcinoma in rats reported by Hanau[1] and the more recent observation of endemic occurrence of carcinoma of the breast in mice by Borrel[2] are well known. Michaelis[3] has likewise recently reported the finding in the course of one year in a single cage of five cases of carcinoma in the mouse. Loeb[4] refers to an article by Cooper, in the *Veterinarian,* on the endemic occurrence of cancer of the parotid and submaxillary glands in the cow, and has himself reported the endemic occurrence of epithelioma of the inner angle of the eye in cattle observed in the Chicago stockyards.

The endemic occurrence of malignant tumors in animals, especially laboratory animals which are confined in cages, and whose diet is controlled, 'and which are under much closer supervision than even the animals of the field, renders observations in regard to the infectiousness of cancer among these animals of much greater significance than many of the similar observations applying to cancer houses and the endemic occurrence of cancer among human beings.

In the light of these interesting observations, the recently reported discovery of the endemic occurrence of cancer of the thyroid in the brook trout hatcheries in Germany by Pick[5] is of the greatest significance. Pick finds that cancer of the thyroid in the various varieties of trout occurs in certain hatcheries in a percentage varying from two to seven, and he refers to an epidemic of this

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[1] Fortschr. d. Med., 1889, vii.

[2] Ann. de l'Inst. Pasteur, 1903, xvii.

[3] Ztschr. f. Krebsforschung, 1906, iv, No. 1.

[4] Centrbl. f. Bakt., 1904, xxxvii.

[5] Vortrag gehalten i. d. Berl. med. Gesell., 1905, October.

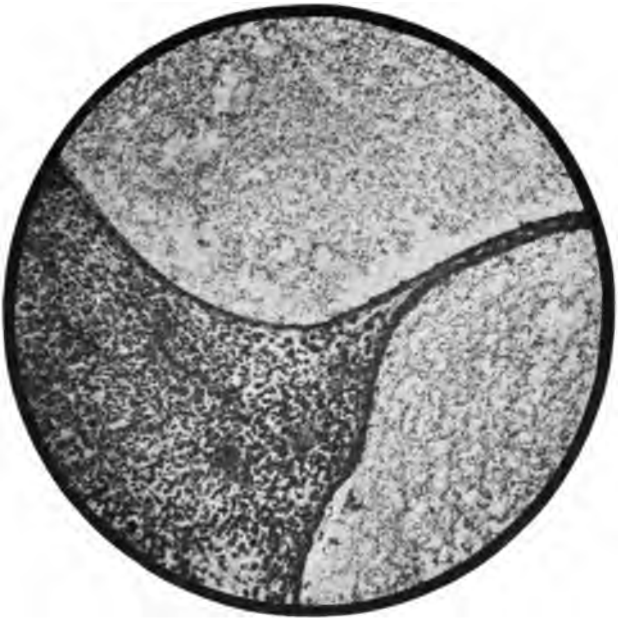

Fig. 1. Microphotograph. × 81.6 (Low power.) Original slide of Dr. Loeb showing character of primary sarcoma of the thyroid in the second Chicago rat. This slide is made from a recurrent mass which developed on the site of the primary tumor. To the left sarcoma tissue which extends into and subdivides a large cyst containing coagulated serum.

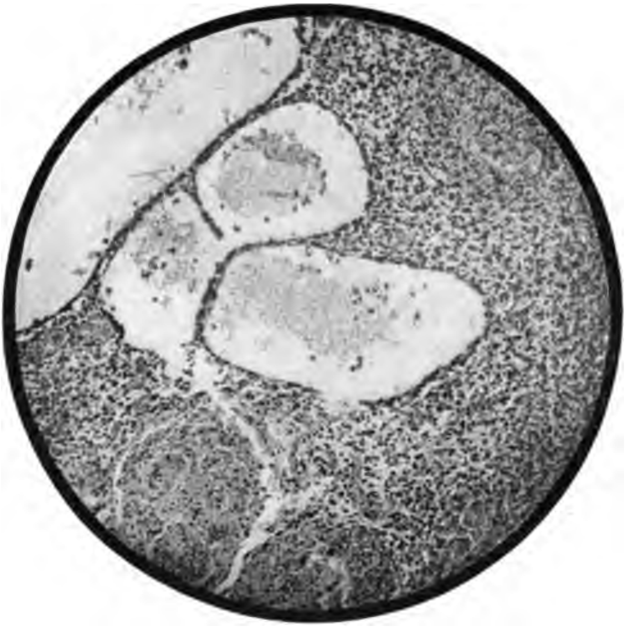

Fig. 2. × 81.6. (Low power.) Transplantation tumor (Dr. Loeb) after 19 days from primary tumor shown in Fig. 1, showing tendency to cyst formation.

affection reported by Bonnet[6] which occurred in the fish hatchery at Torbole on the Gardesee, which, between the middle of February and the end of June, destroyed no less than 3,000 fish. Pick's investigations show that certain hatcheries are entirely free from this affection; that when the fish are affected the disease is confined to individual tanks or pools in which the fish are kept; that wild fish introduced into these ponds for the purpose of replenishing the stock acquire the disease, which observation, to his mind, obviates the necessity of considering heredity an important factor in the development of the infection. His observations establish beyond doubt the nature of the affection, which is true carcinoma, and he concludes that the endemic occurrence of cancer among trout only in certain tanks of hatcheries indicates that the water of these tanks contains the agent which is the cause of the disease. This occurrence he compares with the drinking water origin of struma as it exists in Switzerland, and concludes that the agent, whether chemical, bacterial or protozoon, must be present in the water in which these fish have been kept. During the past two years cases of endemic occurrence of tumors in small animals confined in cages have come under our observation which strongly indicate that cages under given conditions can become infected.

In the case of the sarcoma rats about to be reported, the manner in which the cage became infected would appear to be fairly obvious. The facts pertaining to this observation are as follows:

In 1904, Loeb[4] described the endemic occurrence of primary cystic sarcoma of the thyroid in rats confined in a group of cages in the laboratory of the Chicago Policlinic. The rats in question were found in three or four closely arranged cages which were used to confine a number of rats which were presumably the offspring of a few males and females which had originally been brought into the laboratory. The rats were changed about from cage to cage during the period referred to. In January, 1900, a case of cystic sarcoma of the thyroid was found in these cages. This tumor was transplantable. In August, 1901, a second rat with cystic sarcoma of the thyroid was found in these cages. This was also transplantable. In the autumn of 1903, a third case of cystic sarcoma of the thyroid was found in these cages and this, in the hands of Dr. Herzog, who reported the fact, did not yield results on transplantation.

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[6] **Bayerische Fischereiztg, München, 1883, No. 6, p. 79,** ***et seq.***

The structure of these tumors showed but slight differences. Loeb called attention to the fact that the great similarity of the tumors and the fact that they occurred in a relatively small number of rats which had all, at one time or another, occupied the same cage, indicated that the tumors must owe their origin to a common source. Inasmuch as the rats were all descendants of common ancestors, he was unable to exclude the possibility of the endemic occurrence of these tumors being due to heredity, but felt that the observation just as strongly indicated the possibility of a common source of infection in the cages.

These three cystic sarcomas of the thyroid proved on microscopic examination to possess practically identical histological characteristics. These characteristics in the first and second tumor persisted on transplantation. The clinical course of the disease in inoculated rats was the same. They grew rapidly on inoculation and in the later stages developed cysts of considerable size which contained a straw-colored serum which coagulated on exposure to the air. Areas of necrosis were not infrequent in both primary and transplanted tumors. Loeb demonstrated that the inoculated tumors were derived from implanted cells and that the tumor was a true sarcoma and not an infectious granuloma, in the sense which Bashford[7] has recently employed in attempting to show that the round-celled sarcoma of Sticker[8] was not a true tumor.[9] Dr. Loeb demonstrated sections of both the primary tumors and the inoculation tumors on various occasions before scientific societies, and the universal opinion of all pathologists has been that they were true spindle-celled sarcoma.

In the spring of 1902 Dr. Loeb was engaged in the transplantation of the second tumor and at our invitation came to the State

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[7] Scientific Reports on the Investigations of the Imperial Cancer Research Fund, 1905, part 2.

[8] Ztschr. f. Krebsforschung, 1904, i, No. 5.

[9] In this connection it may be stated that Ewing and Beebe have recently investigated a similar round-celled sarcoma in the dog to that of Sticker and have arrived at the conclusion that Bashford's interpretation is not correct and that both of these tumors are round-celled sarcomas.

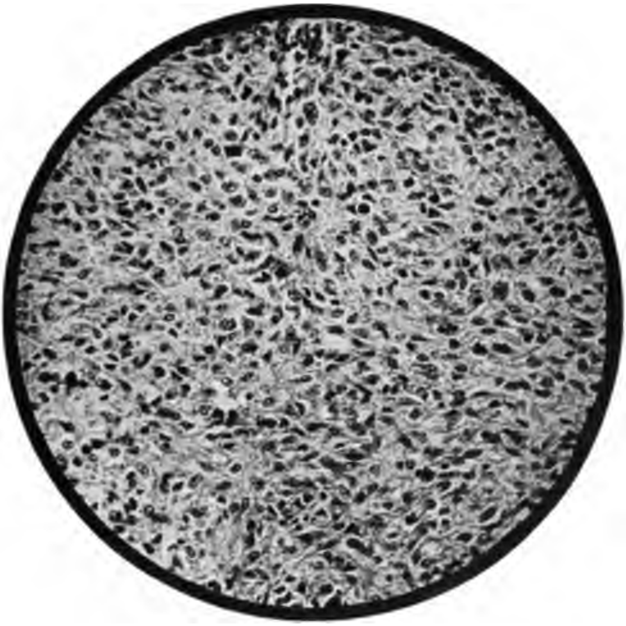

Fig. 3. × 280. (High power.) From section of tumor transplanted from the same source by Dr. Loeb while in Buffalo. This indicates the characteristics of the tumors developing in the rats which occupied the cages during the period of Dr. Loeb's stay at the New York State Cancer Laboratory.

Through the kindness of Dr. Loeb we are able to present three microphotographs. Figures 1, 2 and 3, taken from the second primary cystic sarcoma of the thyroid found in Chicago, from the small intraperitoneal transplanted nodule made by him before coming to Buffalo and a high power microphotograph of a transplanted tumor made in Buffalo from the same source. These when compared with Figures 4, 5 and 6 from similar primary and transplanted tumors from our own Rat B clearly show the striking similarity of these tumors, both primary and transplanted.

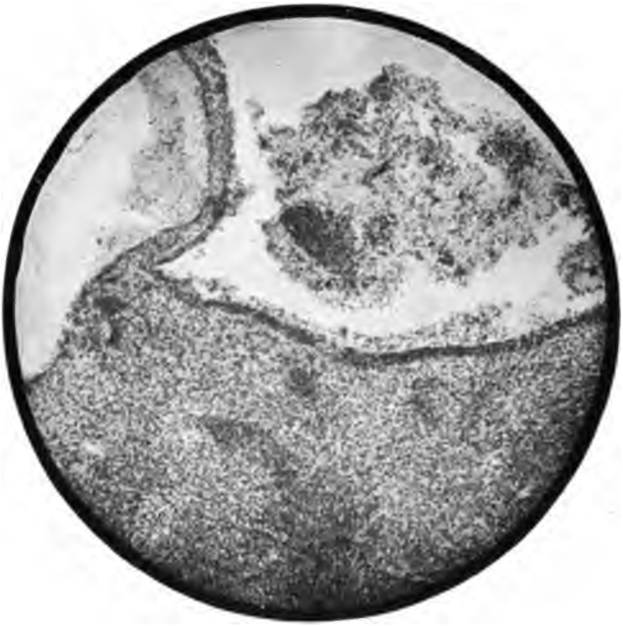

Fig. 4. × 81.6. (Low power.) Section of primary sarcoma of the thyroid in Buffalo Rat 3, showing the sarcoma tissue below extending upward and subdividing large cyst containing disintegrating sarcoma cells and serum ; to be compared with microphotograph. Fig. 1, from Dr. Loeb's primary tumor.

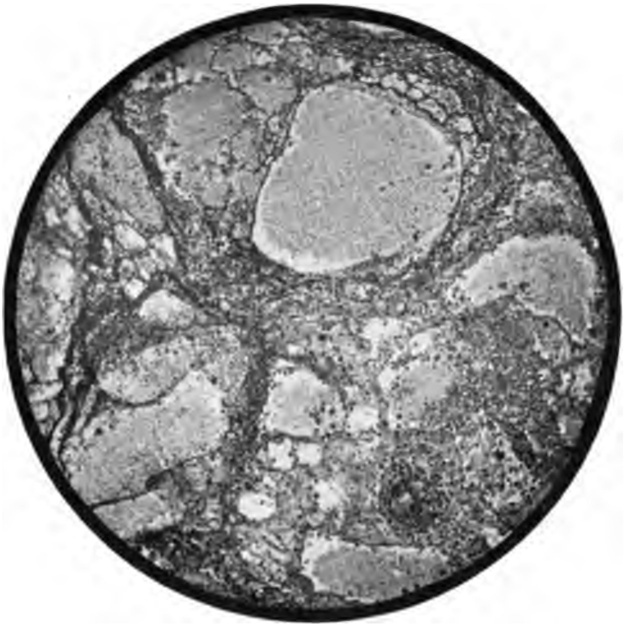

Fig. 5. × 81.6. (Low power.) From cystic portion of tumor transplanted from Rat 3, third generation, showing tendency to cyst formation persisting in transplanted tumors; to be compared with Figure 2 of Dr. Loeb's tumors.

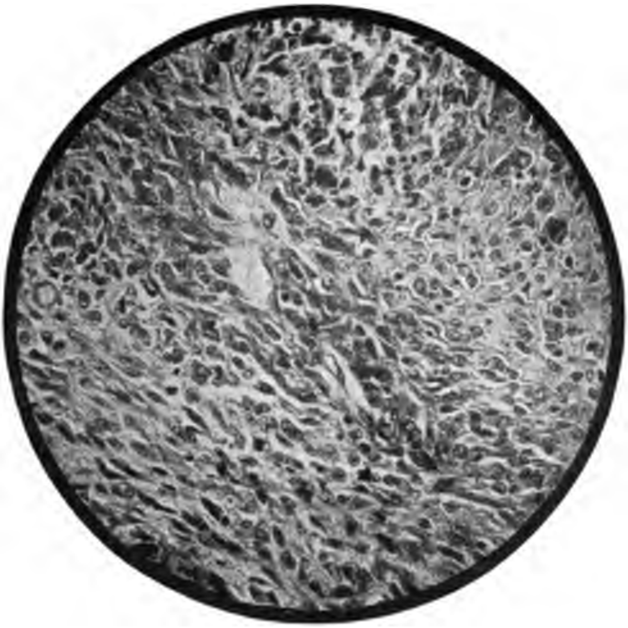

Fig. 6. × 280. (High power.) Sarcoma tissue from transplanted tumor, first generation from Buffalo Rat 3; to be compared with Figure 3 of Dr. Loeb's tumor.

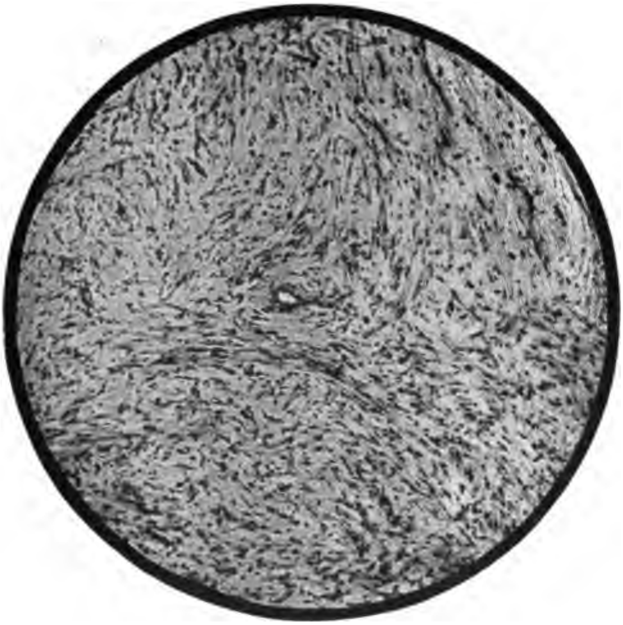

Fig. 7. × 81.6. (Low power.) Section of primary tumor of Rat 1, fibrosarcoma on abdominal aspect. First rat discovered in cage in 1904.

Cancer Laboratory in Buffalo, bringing with him a number of inoculated rats from the second tumor. This tumor had at that time been transplanted through a number of generations. For his accommodation two large cages and a number of smaller ones were made. During the course of his stay in Buffalo these cages all contained, at one time or the other, numbers of successfully inoculated rats. In October, 1902, Dr Loeb went to Montreal, taking his rats with him. Some inoculated rats were left in the cages but none of these developed tumors, and by December, 1902, the remainder were disposed of. *During the period from December,* 1902, *until the summer of* 1903, *there were no rats in the laboratory.* The smaller cages were placed in the hot-air sterilizer and sterilized; the two larger, being too bulky for this procedure, were brushed out and stored in the back part of the animal room, where they remained unused until the summer of 1903, when they were employed for a number of rats brought into the laboratory for purposes of study other than tumor implantation. These rats were killed at various periods and on the conclusion of the work perhaps a dozen rats were left in the two large cages; in one cage four rats, in the other six or eight. This entire lot of rats was obtained from a totally distinct source from those which Dr. Loeb had employed.

In July, 1904, in one of the two large cages, which had not been subjected to sterilization after Dr. Loeb's departure, and which at that time contained four rats, a rat was discovered with a large tumor on the right abdominal aspect and a smaller tumor adjacent to it in the axillary region, about the size of a hazelnut. The large tumor was movable, dense, and about the size of an English walnut. The rat appeared to be in good condition and on July 12th, the large tumor was removed by operation. It proved on section to be a slow-growing fibrosarcoma. Portions of the tumor were inoculated into a number of rats but none of these subsequently developed tumors. There was no local recurrence of the tumor at the site of the operation, and on the death of the rat some months later the smaller nodule in the axilla was found not to have changed in size. The remaining rats were removed from both cages.

Eight rats from an entirely new source were now placed in the cage in which the tumor had appeared. In the other cage of similar size were placed six or eight rats from the same source as the rats now placed in the infected cage. Both cages were now removed to the basement. They had previously been in the animal room of the laboratory, which is on the fourth floor. The conditions in the basement were not essentially different, except that the room was not so well heated and possibly more damp. The rats remained undisturbed in these cages in the basement of the laboratory from August, 1904, until October, 1905. At that time three adult rats were in the cage in which the first tumor had been found. They were the survivors of the eight which had been originally introduced into the cage in August, 1904, five having died of intercurrent disease (tuberculosis), one three-quarters grown female which had obviously been born in the cage and a litter of ten half-grown rats, not over six months old, all of these the offspring of the adult rats originally placed in the cage. Of the three adult rats, two were males, one a female. The two males were found on examination each to have a large tumor. One of these had a tumor located on the right side of the abdominal aspect directly behind the right fore-leg. It was about the size of a fifty-cent piece, 1 cm. in thickness. The other male adult rat possessed a tumor the size of a walnut in the thyroid region. This tumor, because of its great size, embarrassed the respiration of the rat and it died October 6th. This rat is known as Buffalo Rat. 1. The notes of the autopsy are as follows:

The tumor occupies the thyroid region and extends from the chin to the sternum. It is nearly spherical. It measures 35 mm. in the long axis by 45 in the transverse axis. The skin over the tumor is thin, of bluish appearance, but everywhere perfectly movable. The tumor is fixed at its base, is nowhere adherent except in the median line in the deeper portion of the neck. The greatest thickness of the tumor is 35 mm., its weight is 19.8 gm. On dissecting up the tumor it is found to be distinctly encapsulated, easily dissected from the skin, with areas of deep purple mottling on its superior surface and injected blood vessels in the capsule. Toward the base the tumor is of lighter color and greater consistence. It is easily dissected down to a point where its anatomic relations correspond with the thyroid. There is no evident compression of the trachea.

On opening the abdominal cavity the mesentery is found to contain a considerable amount of fat. Intestines are collapsed and pale. The liver is greatly enlarged and presents a typical nutmeg appearance.

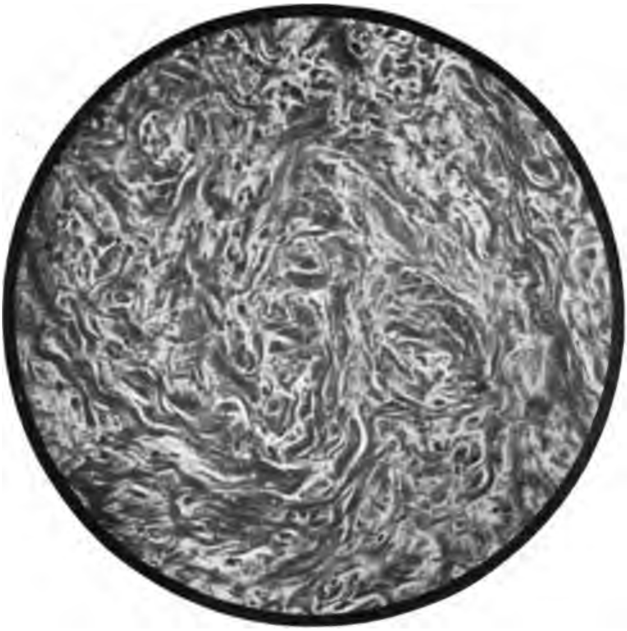

Fig. 8. × 280. (High power.) Primary tumor of Rat 2, showing fibrous character of primary tumor.

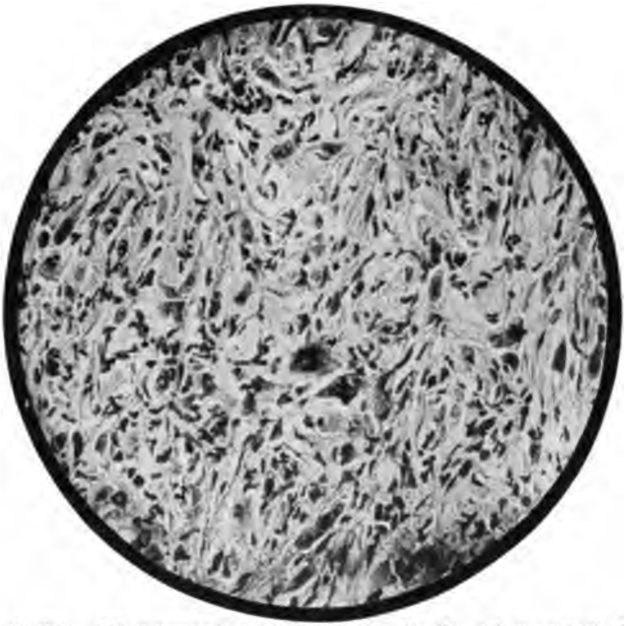

Fig. 9. × 280. (High power.) Buffalo Rat 2. Section of metastasis in median fissure of liver, showing sarcomatous character of tumor and the tendency to formation of intracellular fibrous substance.

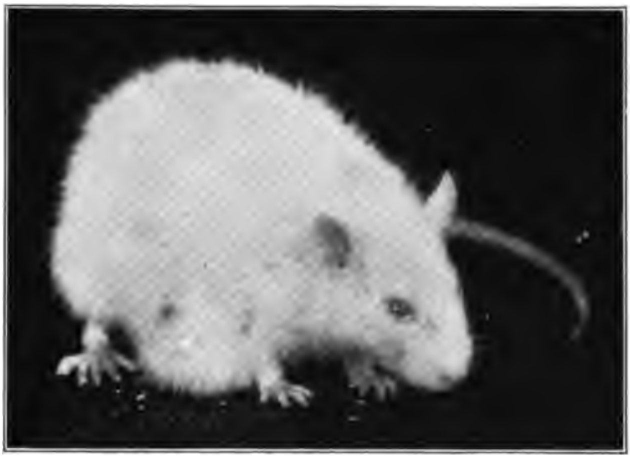

Fig. 10. Buffalo Rat 1. Large fibrosarcoma of abdominal aspect. Found in cage, July, 1904, which had contained Dr. Loeb's rats in 1903.

On examination of the lungs the lower lobe on the left side is found to be in a state of consolidation, with mottlings of deep red, on which are distributed well-defined areas of pale white. The upper middle lobe on the right side shows the same condition. The left adrenal is enlarged, hyperemic and nodular in appearance, is very friable on removal. The kidney is soft and large. The right adrenal is small and of normal appearance. Kidneys on both sides present the same appearance, somewhat enlarged, dark red, granular surface.

The liver on section presents the characteristics of high-grade fatty degeneration and cyanosis, typical nutmeg appearance. The spleen and pancreas appear to be of normal size and appearance. The retro-bronchial lymph nodes are not enlarged, the mesenteric lymph nodes are also not enlarged. The enlarged left adrenal on section is of dark red color, friable and soft.

On opening the tumor the left half is found to consist of a thick-walled cyst containing clear, straw-colored fluid which, on exposure to the air, coagulates. The wall of the cyst is pinkish white, the interior smooth and glistening. The right lobe of the tumor consists of friable, pinkish white tissue, with large areas of hemorrhage. The entire tumor, after removing specimens for microscopic examination, was ground up with salt solution and injected into rats.

Histologic examination of this tumor shows it to be a spindle-celled sarcoma, containing frequent cysts. Here and there in the primary tumor are remnants of thyroid epithelium, arranged in the form of irregular nests, but showing no evidence of proliferation. The typical sarcomatous structure is well-defined at the margins of the large cystic cavity, which forms the bulk of the tumor. After removing sufficient material for hardening, the remainder of the tumor was broken up into small pieces, under aseptic conditions and used to inoculate ten white rats and six parti-colored rats, by placing small pieces of the tumor beneath the skin with a sterile trocar. Four rats were also inoculated with the serum obtained from the large cystic cavity. The result of the first attempts at inoculation yielded four tumors, all in the white rats, none appearing in the parti-colored; two of these in white rats inoculated with fragments of tumor through a trocar, and two from the four rats inoculated with the serum of the cyst cavity.

From the four tumors thus obtained the tumor has now been carried to the tenth generation of successful transplantation. In the course of inoculation we have had one or two tumors develop in the parti-colored rats, and although the percentage of successful inoculations is not as yet very high, the virulence of the tumor and the percentage of successful inoculations appear to be increasing.

Sections from the tumors obtained by implantation present the histologic characteristics of the primary tumor. These tumors grow to great size and in the later days of the disease rapidly form cysts, by taking on large quantities of serum. The behavior of these tumors is in every way like the implanted tumors which Dr. Loeb had in the laboratory in the spring and summer of 1902. These histologic characteristics of the tumor, both in the primary and implanted tumors, differ from Dr. Loeb's primary and his secondary implantations, only in minor details. They show the same characteristic structure of sarcoma tissue into a network surrounding the cysts. The only point of difference is an occasional appearance in the arrangement of the cells which suggests alveolar sarcoma, but most of the tumors are of the simple spindle-celled variety.

The second rat, Buffalo Rat 2, found in the cage in October, 1905, was in good condition. On October 23d this rat was subjected to an operation at which the tumor was removed. At the operation it was found that the tumor extended beyond the median line. There was a small nodule detached from the tumor on the thoracic aspect and this was left *in situ,* after being cut in several directions with sterile shears. The tumor weighed 19.8 grams. It presented the macroscopic appearance of a fibrosarcoma. It was transplanted into twenty white rats. On section it proved to be a fibrosarcoma, identical in appearance with the tumor in the first rat discovered in the cage in the summer of 1904. Attempts at transplantation were all unsuccessful, no tumors developing in the twenty rats inoculated. The rat lived until January 10, 1906, when it was found dead in its cage.

The autopsy showed that the nodule which was left in place at the operation in October had increased in size, had infiltrated the surrounding tissues, and proved on section to be somewhat softer than when incised at the time of operation. The liver showed marked evidence of fatty degeneration. In the median fissure between the right and left lobes was a large metastasis of irregular shape, somewhat larger than a hazelnut. It involved the structure of the liver and on section was pinkish white in

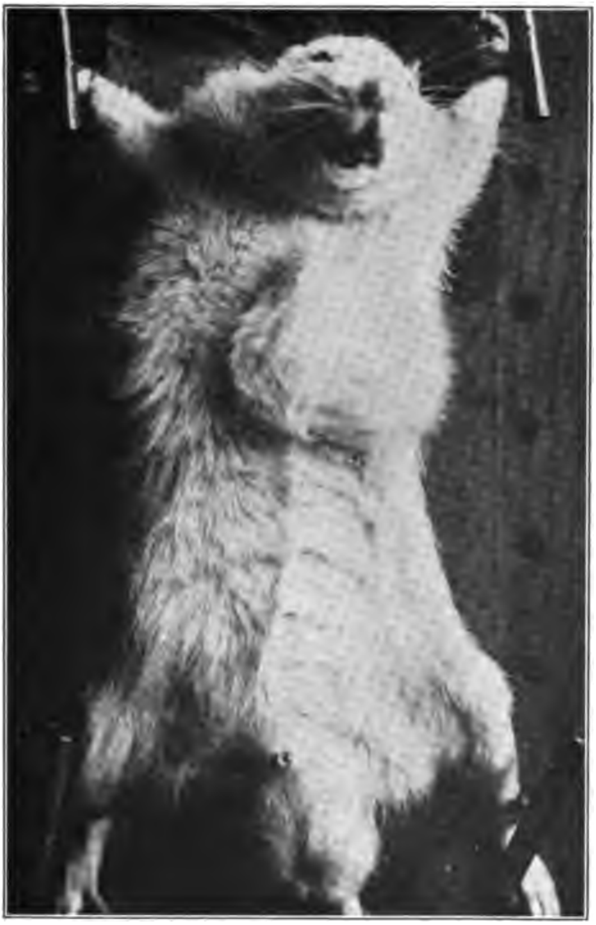

Fig. 11. Buffalo Rat 2. Large fibrosarcoma of abdominal aspect. Developed tumor in same cage and was first observed, October, 1905.

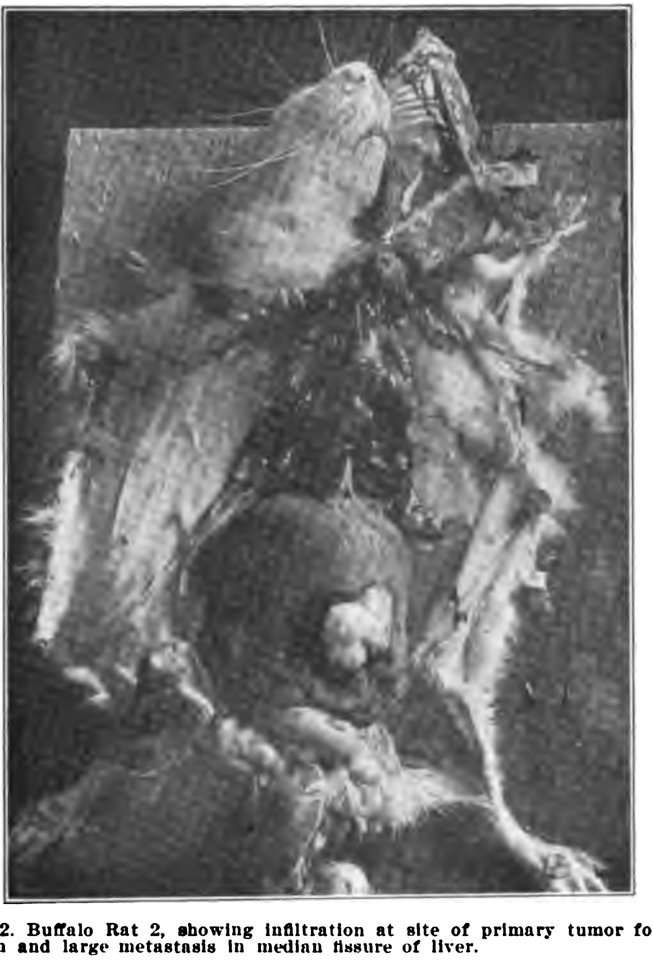

Fig. 12. Buffalo Rat 2, showing infiltration at site of primary tumor following operation and large metastasis in median fissure of liver.

color. On elevating the liver numerous mesenteric metastases, one somewhat larger than a pea, and numerous metastases scattered over the peritoneal surface of the intestines, spleen and mesentery, were found. The tumor material and organs of the animal appeared to be badly contaminated and transplantation experiments were not undertaken. Histologic sections of the metastases of this tumor showed that it had assumed the characteristics of a rapidly-growing spindle-celled sarcoma.

During the period in which these three tumors were found in one cage, in the State Laboratory, no tumors appeared in the control cage, in which there were kept approximately an equal number of rats during the entire period, nor in the other cages in the laboratory, although during the greater part of the time there were no less than 100 rats in various cages. No tumors developed in the smaller cages which were used by Dr. Loeb, but which were subsequently sterilized.

Sarcoma of the thyroid in the rat appears to be a rare affection. We have been unable to find reports of this disease except in the case of the three primary tumors observed by Dr. Loeb. For the purpose of ascertaining how frequent this affection might be, there was sent from this laboratory to 325 breeders of small animals in the United States a postal card on which was printed a photograph of Rat 1, with the inquiry as to whether or not these dealers had observed any case of a rat with a similar tumor in the neck and offering $25 reward for each animal so affected. The inquiry was also made as to how many white rats the dealer had observed or handled during the preceding three years. Answers were received from fifty-seven dealers, who reported having had approximately 20,000 white rats in their establishments. All but one or two stated they had never seen anything like the photograph of Rat 1, and none were able to give a description which clearly indicated that they had observed anything but abscesses or indefinite swellings in other regions in their animals.

A summary of the facts in this observation is as follows: There were seven rats in all which survived in the infected cage. In the first instance four rats remained in the cage for a period somewhat over a year. One of these developed a large fibrosarcoma on the abdominal aspect. In the second case three rats survived in the cage a period of fourteen months. Two of them developed sarcomas, one, Rat 1, a primary sarcoma of the thyroid, Rat 2 a fibrosarcoma on the abdominal aspect. The histologic findings showed that the first fibrosarcoma and the second fibrosarcoma were neither transplantable. They presented the same histologic characteristics. The first one was totally removed and did not recur. In the second a nodule was left *in situ* and the animal died with abundant metastases of rapidly-growing spindle-celled sarcoma. These tumors must then be looked on as malignant tumors, although they were not transplantable and the first one did not recur. The thyroid tumor presents all the essential characteristics of Dr. Loeb's sarcoma of the thyroid, implanted rats from which had previously occupied this cage.

It would be natural to attempt to separate the two fibrosarcomas from the transplantable and rapidly-growing sarcoma of the thyroid, and we should have expressed a possible doubt that they were derived from the same form of contagion, were it not for the recent experiments of Ehrlich and Apolant, which have demonstrated beyond doubt that even a carcinoma can, under given conditions, lead to the development of a sarcoma in connective tissue immediately adjacent to it. For this reason it would seem an unnecessary refinement to consider that the three cases of sarcoma, although in different regions in the animal and somewhat different in histologic details and experimental characteristics, were attributable to other than a common source. In the light of Borrel's[2] observations on the endemic occurrence of carcinoma in mice, in which he succeeded in tracing the origin of twenty mouse tumors to one cage, it would seem that the source of infection in this case is vested in the cage in which these rats were contained. That we may positively exclude heredity is owing to the fact that the rats which occupied the cage from 1903 to 1904 were derived from a distinct source in a widely distant city from the rats which occupied the cage from 1904 to 1905. That local environment is not the essential factor is shown by the fact that the cage occupied space in the animal room on the fourth floor of the building from 1903 to 1904; from 1904 to 1905 in the basement of the laboratory.

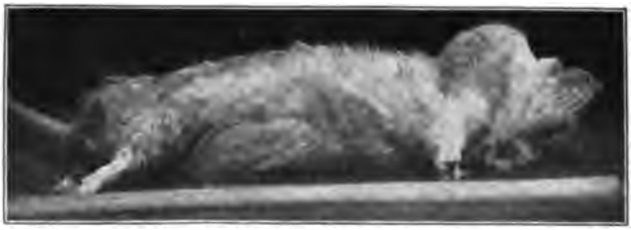

Fig. 13. Buffalo Rat 3. Primary cystic sarcoma of thyroid developed in cage at the same time with Buffalo Rat 2, October, 1905.

The most logical explanation of the manner in which this cage became infected with the contagion of sarcoma is found in the fact that it was used in 1902 by Dr. Loeb, who kept in it at that time rats inoculated with the second cystic sarcoma of the thyroid discovered in Chicago. It is to be noted that a period of three years elapsed from the time of its employment by Dr. Loeb to the development of the primary cystic sarcoma of the thyroid in 1905. From this it would appear that the contagion of sarcoma in the rat is extremely persistent as to time, extending over a period not less than one-third the life of the rat.

It is of interest to note that two of the three primary sarcomas of the thyroid in Chicago were first detected in the summer or autumn; that all three of our rat tumors were likewise detected during the summer or autumn, and that in both cases a period of approximately one year intervened between the development of the different tumors. From this it seems probable that the incubation period, or the period in which a rat must be exposed to the contagion, must extend over a number of months. Our cage at present contains a number of rats which have been undisturbed in it since October, 1905. To these have also been added a number of rats during the past month. The conditions of the cage are unchanged. These rats have been obtained from another source distinct from that of any of the rats thus far employed, and a further report will be made after sufficient time has elapsed to make it probable that tumors will or will not develop.

Our second observation relates to the endemic occurrence of cancer among mice in the establishment of a dealer who has been engaged in the raising of these animals for several years, in Springfield, Ohio. In view of the completeness of the data placed at our disposal, we consider that this case affords the most striking illustration of cage infection thus far reported.

One of us visited the establishment in question June 8, 1906, in the company of Dr. Rand, of Springfield, who heard the entire statement made by Mr. Landes, the owner of the establishment and a man of intelligence. The statement which he gave us regarding the occurrence of these tumors, many of which came under our own personal observation, appeared to be perfectly logical and entirely free from discrepancies. In the course of the previous year Mr. Landes had sent this laboratory six white mice with spontaneous tumors, which proved on microscopic examination to be adenocarcinomata of the breast. They were all in females and all located on the abdominal aspect. We found on inquiry that Mr. Landes recognized the fact that the source of these tumors was one old cage, built of wood, one end of which was screened off with netting. He stated that the cage was three years old and that it had contained for three years an average of about 100 old mice. He estimated that these mice bred to such an extent that he was able to get between one and two thousand young ones out of this cage, annually.

The history of this cage is as follows: It was built in July, 1903, at his place of business, which was then at the corner of Shafer and Columbia streets, two and one-half miles distant from its present location. It was kept in a barn, the boards of which were poorly matched, and the place was cold and windy in winter. It remained nearly a year in this barn and contained during this period about 100 mice. During the course of the winter he found one or two mice with tumors in the cage. In April, 1904, he moved to the corner of Light and Cedar streets, two or three squares from the first location, and the cage was kept in a large coal shed, which was warm and comfortable. It remained from April to November, 1904, in this locality and during that period he removed from the cage twenty-five to thirty mice with tumors. In November, 1904, he moved to his present location, two and one-half miles distant from the first two mentioned. Before the cage was removed from Light and Cedar streets he observed twelve mice at one time with tumors, and for the purpose of ridding himself of this unfortunate development of tumors he decided to change entirely the stock in the cage. All the mice which had occupied the cage were removed and twelve adult, healthy mice, ten females and two males, were imported from Washington, D. C., and introduced into the cage, which was placed in a small, detached outhouse, at least fifty feet distant from the present location of the cage. During the course of this winter three or four tumors developed.

Since the spring of 1905, the cage has been in a large room thirty by fifty feet in size, which was previously a dance hall and it now stands on a table six to seven feet from a window where the conditions of light and ventilation are excellent. It previously stood in a different position in the room about twenty feet from its present location. During the last year he has removed between twenty-five and thirty mice with tumors from the cage, several of which have been sent to us. Owing to a misunderstanding on his part he had the idea that only tumors between the front legs were what we desired, and those which appeared on the flanks or lateral aspect of the abdominal region he killed. At the time of my visit one mouse was in the cage with two large tumors on the right abdominal aspect. He pointed this out as an example of the kind of tumors which he thought we did not require. He states that he has never seen a tumor on the back of any mouse. He thinks they were mostly females, and in several instances when he examined them as to their sex he found they were females. He has never seen a male with a tumor. The tumors have frequently grown to great size.

Besides the old cage, his establishment contains twelve or fifteen other cages of similar construction. One of these is two years old, the remainder one year old. They are regularly stocked from the old cage. His custom is to remove from the old cage twelve or more females with one or two bucks and place them in the new cages and allow them to remain there until each cage contains approximately 100 mice. The half and three-quarter grown offspring are removed and sold. In the cage which is now two years old he has during the past year observed four to six mice with tumors. So far no tumors have appeared in mice in other cages in the establishment. He remembers having seen one or two tumor mice in some of his old cages in his previous establishments, but these cages were always stocked from the old cage already described, which seems to have been the source of all his operations, and which he referred to as his incubator. The cage was purchased by the laboratory and brought to Buffalo with the mice in it. On reaching the laboratory it was found to contain three mice with large tumors. The interior of the cage is dark and damp, incrusted with excreta and presents a generally unhygienic appearance. Examination on the date of sending this manuscript for publication, August 3, shows that the cage contains twenty-eight adults and perhaps twice as many half-grown and young mice. On the floor of the cage is the carcass of a mouse which has apparently been dead some hours with a large tumor on the abdominal aspect. This is ulcerated and shows evidence of having been gnawed. A second mouse with a tumor the size of a large hazelnut protruding between the hind legs and evidently springing from the posterior part of the mammary tissue is also found. The skin over this tumor is adherent and the tumor is evidently far advanced.

Briefly stated, the facts in the above case are as follows: A cage has been discovered in which upwards of sixty spontaneous tumors have occurred in the course of three years. The fact that the location of the cage was frequently changed and the stock entirely renewed on at least one occasion without any permanent interference with the production of tumors, makes it apparent that the cage itself was the source of infection.

Besides these observations which point directly to the cage as the source of infection, the endemic occurrence of cancer among mice in breeding establishments is well known and is illustrated to a remarkable degree in our own experience. For instance, from January, 1905, until the present time, this laboratory has had a standing reward of $25 for any small animal affected with cancer. This offer, as already stated, was sent to 325 dealers in pet animals. It is possible that many of them have not appreciated the significance of the offer, or have overlooked cases of cancer in their stock, but during this period we have had constant business relations with seven dealers in different parts of the country, from whom we have purchased large numbers of mice, and who, we feel perfectly certain, have fully appreciated the monetary value of cancer mice. From one of these dealers we have received no less than eighteen female mice with cancer of the breast; from a second dealer, five of the same nature and sex; from two other dealers, one each, and from three from whom we have had repeated shipments of mice, none whatsoever. From one of these dealers from whom we have received in the last two years not less than 1,200 normal mice, we learn that he has never, in his own stock, seen an example of cancer of the mouse, but that he was able to recognize the affection was shown by his having secured for us from another dealer a single specimen.

These figures conclusively indicate that in certain breeding establishments cancer in white mice is endemic. The condition of affairs in the breeding establishment of a dealer in Massachusetts is interesting when compared with that of the Springfield dealer where the evidence pointed to a single cage as the source of infection. This Massachusetts dealer has shipped to us, in all, eighteen cancer mice, in lots of nine, four and five, and it is of great interest that the tumors in all of these mice were of relatively the same size. On inquiry as to where the tumors had developed, if they could be traced to a given cage or group of cages, we were notified that the mice were scattered indiscriminately through the entire establishment. This condition of affairs was so interesting that we made a trip to Massachusetts to personally inspect the premises, whereupon it was found that this dealer, in order to combat infections and contagious disease had her stock distributed among a large number of small boxes. These mice were moved about from box to box and the different families were regularly subdivided and used for the purpose of forming new families. This practice is entirely different from that of the Springfield establishment and easily explains the general distribution of the tumor mice through the different breeding boxes. The fact that the tumors have developed in lots of half a dozen or more at one time and that in the various shipments to us the tumors have been of relatively the same size, suggest that small epidemics have occurred at frequent intervals. The attempt to trace the origin of the infection to any particular box in the establishment is, of course, under the present system, impossible, and it is not improbable that this method has led to the dissemination of the contagion through a large number of the boxes.

The foregoing observations indicate that both sarcoma in rats and carcinoma of the breast in mice must be looked on as contagious, and when considered in conjunction with the classical observations of Loeb[4] and Borrel,[2] in which, however, it was impossible entirely to exclude the factor of heredity, should lead us to pay more serious consideration to the interesting statistics constantly accumulating which show the probable infection of the surroundings of human cancer cases in so-called "cancer house." It should also lead to earnest consideration of the desirability of sterilizing the dressings of cancer cases and the complete sterilization of rooms which patients have occupied, and it should, at least, to no inconsiderable extent, offset the recent statement of Hansemann, that we have no right to add to the difficulties of the cancer patient by the unnecessary suspicion that he is suffering from an infectious disease. It should tend to combat the belief among pathologists that there are no grounds for even suspecting an infectious factor in malignant tumors.

EIGHTH ANNUAL REPORT

of the work of the

CANCER LABORATORY

of the

ew York State Department of Health

Conducted at the

GRATWICK RESEARCH LABORATORY

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CANCER LABORATORY

BUFFALO, N. Y., *February* 1, 1908.

DR. EUGENE H. PORTER, *Commissioner of Health, Albany, N. Y.:*

MY DEAR SIR: — I herewith transmit to you the Eighth Annual Report of the New York State Cancer Laboratory of the Department of Health, for the fiscal year ending October 1, 1907.

The last year has been one of rapid advance in cancer research, and we have again witnessed, as in the two preceding years, the advance of this reseach along the lines which this laboratory has been following since its foundation. In June of the present year the State Cancer Laboratory will have completed its tenth year of existence. It is perhaps well that at this time we should review the work of the laboratory for the first decade, and attempt to determine the relation of this institute to the modern cancer research in which it has taken a prominent part.

The first three years after the foundation of the laboratory were occupied in attempts to define the cancer problem and to determine which particular lines of research were the most promising. From the very beginning we were impressed with the belief that only through experimental methods could progress be expected. It can be fairly stated that in the two decades preceding 1898 no progress whatsoever had been made in the solution of the etiology of cancer, and that the purely histological methods in vogue at that time gave no immediate promise of assistance. The first three years of work were divided between working over the observations of other investigators and the introduction of the experimentation with animals. A long series of experiments was conducted to see if it was possible to transmit human cancer to animals. These experiments were generally negative, but in two instances animals which were inoculated with human cancer developed neoplasms under conditions which make it possible that the growths were due to the inoculation. Extensive attempts have been made to accomplish the same thing in other quarters, also with very meager results, and it can be generally stated that human cancer is not transmissible to animals by direct inoculation; certainly not with any regularity by the means which have been thus far employed. Dagonet and Mauclaire (Archives de medicine experimentale 1904, No. 5) and Lewin (Deut. med. Wochenschrift, 1905, No. 33) report the success of the transmission of human cancer, in the first case into rats, and in Lewin's experiments into a dog. In the light of the now well established facts regarding the transplantability in animals of the same species, it must be conceded that cancer is not directly communicable by contact from human beings to animals, or from animals to human beings; but in the light of the difficulties which are frequently encountered in transplanting primary tumors even into animals of the same species, we must realize that we do not properly understand all the conditions underlying transplantability, and that under unfavorable conditions this form of transmission of cancer must be out of the question. Ehrlich has shown that very active mouse cancer can be transplanted with some success into rats. These tumors usually grow but a few days in the rats and have then to be transplanted back into mice to continue the propagation. In one instance, however, a mouse tumor has grown to considerable size and persisted for over a month in a rat. Guthrie McConnell has also shown that human cancer can apparently maintain its vitality under favorable conditions for several months in animals, without growing.

During the first period of our work a large number of inoculations of human cancer into animals showed that the animals so inoculated died with astonishing regularity, not of cancer, but through some curious and obscure form of marasmus, in which the animals lost weight, became anæmic and presented a picture not unlike the so-called cancer cachexia. These results were not due to contamination with bacteria, and it is possible that there is an agent in human cancer which, although incapable of continuing the growth of human cancer in animals, may initiate a process not unlike the terminal phases of the disease terminating in the death of animals so inoculated. These experiments should be taken up again and carried to a final conclusion. It was also determined that the enlarged lymph nodes taken from the animals which had died in this way, when introduced into healthy animals, were capable of repeating the obscure process referred to, the animals dying in a shorter period of time than in the first instance.

During the first period of our work we were largely engaged in attempting to determine with the microscope, the significance of certain inclusions in the cancer cells and certain free bodies found among them. In the Third Annual Report of the laboratory, we definitely committed ourselves to the parasitic theory of cancer, believing at that time that we had found the specific organism of cancer, and in the Fourth Annual Report of the laboratory, we attempted to justify our findings by comparison of the supposed organism with other known organisms of similar appearance. During the last five years we have not been able to bring any conclusive proof of the parasitic nature of these bodies, and realizing that without extensive collateral knowledge this would probably be impossible, we have directed our efforts in the last five years of work, to the collection of evidence tending to show in a general way that cancer is an infectious disease.

In the Third Annual Report of the laboratory, we were able to publish a carefully prepared and comprehensive statistical study, not only of the distribution of cancer in Buffalo (by Dr. Lyon), but also a study of the local distribution of cancer in a small town in Western New York. Both of these studies tended to show, in the first instance, a curious distribution of cancer, perhaps most easily understood when cancer is viewed as an infectious disease; and in the case of the small town of Western New York, only two explanations being possible for the increased number of cancer cases in this locality: first, heredity, and second, infection by contact or through indirect means. Since the publication of these papers, the discovery of cage infection among animals and the accumulating evidence of the endemic occurrence of cancer in localities the world over, have reached a point where it is highly probable that heredity is a contributing influence, and in the light of the readiness with which tumors can be transplanted among animals, it must be conceded that contact probably explains a certain number of these cases, whereas the remainder tend to support the belief in the infectiousness of cancer. We have detailed at length in our various reports evidence relating to infected cages, animals introduced into which have acquired the disease. We have under observation a breeding establishment in New England, from which we receive several white mice every month with cancer of the breast. The detailed facts relating to this observation should prove absolutely convincing that cancer under given conditions is endemic.

In 1900 there was added to the staff of the laboratory, Dr. G. H. A. Clowes, a trained physical and biological chemist, who has conducted extensive, independent chemical researches on cancer. In the Fifth Annual Report he published a series of articles on the metabolism of cancer and investigations into the reliability of certain chemical methods used in the diagnosis of cancer of the stomach. Dr. Clowes also initiated the immune work on animals, the pathological and chemical work of the laboratory having united in this branch of research. In 1903 Professor G. N. Calkins of Columbia University accepted the position of consulting biologist to the laboratory and we have had the advantage of his advice in matters pertaining to his branch, and he has published several important articles in the reports of the laboratory.

In 1903 Dr. Leo Loeb spent part of the winter at the laboratory, conducting his new classical transplantation experiments with sarcoma in rats. In 1904 the laboratory came into possession, through the kindness of Professor Jensen of Copenhagen of the well known transplantable mouse cancer discovered by that investigator. Through this acquisition we were at once able to turn our attention to phases of the cancer problem which had attracted our interest from the very beginning of our experimental work. We were anxious to apply facts relating to immunity, firmly established in many of the infectious diseases. Believing confidently that cancer would prove to be an infectious disease, these experiments became naturally of the first concern to us, and with the opportunity afforded by the acquisition of animals with cancer, we have been able to demonstrate certain facts, which have been reported upon in detail in the annual reports of the laboratory. Some of these facts are fundamental and have now been confirmed in many quarters both in this country and in Europe. We were able to report to you in the Sixth and Seventh Annual Reports of the laboratory, that cancer is a curable disease; that animals recover spontaneously from cancer, are then immune, and that their blood possesses immune qualities, being injected into animals with small tumors frequently causing the disappearance of the same and the cure of the animals, and in the later stages of the disease markedly improving the condition of the animal and inhibiting the advance of the disease.

Dr. Clowes also showed that cancer material in test tubes treated with the blood of normal animals was inhibited in its growth, and that cancer material treated with the blood of recovered animals would, under suitable conditions, entirely lose its infectiveness, and that the number of successful transplantations accomplished after such treatment was reduced very materially.

Our first results relating to immunity were obtained in 1904 and published in January, 1905. In 1906 Professor Ehrlich of Frankfurt published results along similar lines, his methods of procedure being somewhat different from ours, he having succeeded in immunizing animals by inoculating them with tumors of low infectivity, and in this way raising the resistance of the animals so that they could withstand doses of more active materials. Ehrlich and his students have rather questioned our belief that the immune factors could be definitely located in the blood, and Bashford, an English investigator, who has strongly questioned our facts, has later confirmed them. He still declines to accept our interpretation of our results, although he succeeded in immunizing animals with the blood of normal animals, being a variation of Dr. Clowes' experiments in treating cancer material in the test tube with the blood of normal animals. The investigations and observations of Crile and Beebe, working under the Huntington Research Fund in New York, greatly strengthen our position. These observers, who have been working with a round-celled sarcoma in dogs have succeeded in curing animals in the last stages of the disease by transfusing diseased dogs with part or all of the blood of recovered dogs. These results have been obtained with such striking regularity, and as they are in principle an amplification of our original observations, we see no reason for altering our point of view that the immunity acquired by animals after recovery from cancer finds an important, if not its main medium of activity, in the blood. Lewin working in the Royal Institute for Cancer Research in Berlin, with cancer in rats, has recently confirmed these observations in full.

The establishment of a definite immune process in cancer can only mean that the principles of this discovery must some day prove applicable in some form or other to cancer in human beings. It is, however, impossible to state what difficulties will have to be overcome, what fields explored or what disappointments met, before such a desirable end may be attained. Professor Ehrlich, in a public address in Berlin, on the occasion of announcing his results, which are in principle but slightly different from ours, made the statement that the serum-therapy of cancer was only a matter of time.

During the last five years the experimental investigation of cancer has broadened to such an extent that many investigators in different parts of the world are now pressing into this field, national institutes and societies have been founded and an international society for the investigation and combatting of cancer is in process of formation. To-day the outlook in cancer research is not only encouraging, but distinctly promising. If one calls to mind the pessimism which reigned ten years ago regarding cancer, and realizes to-day how differently the whole disease is viewed, how actively investigators are hopefully working upon the problem, and how promising is the outlook, one must concede that the results obtained are far greater than we could have possibly promised ourselves at the time this laboratory was founded by the State.

In this connection I desire to call your attention to the status of this institution. It stands to-day the first State laboratory or institute founded for the special investigation of cancer. Within the ten years of its existence is encompassed all of the modern discoveries which have placed cancer research where it is to-day. This institute has contributed to and borne its share of this research. It has lived a precarious existence; the members of its staff have gone on from year to year, through years of depression, criticism and disappointment, until to-day it has established for itself a position as a pioneer institute, recognized as a definite factor in cancer research the world over. Beginning with two rooms, kindly placed at the disposal of the work by the University of Buffalo, the public-spirited citizens of Buffalo have erected for its exclusive use a handsome and well equipped laboratory, at a cost of more than $50,000. This institute has been placed at the disposal of the State so long as it shall maintain this work. The scope of our work has increased from year to year, as the possibilities opened by our observations and progress have developed. Starting with an appropriation from the Legislature of $10,000 the first year, with the development of our activities we found that we could profitably utilize $15,000 and with the still further development of our work we have for the last three years requested the Legislature to increase our appropriation. The opportunities to-day for advance are so great, the promise of the ultimate solution of this problem is so hopeful, and the devastation which the disease is carrying on among human beings is so terrific, that the Legislature should increase our opportunities and support our hands as much as possible. As you are well aware, the members of the staff of this laboratory have showed their obligations to this work, not from a financial standpoint, but from one of obligation to this work and to humanity. A review of our accounts since the establishment of the laboratory will surprise many. To-day we are heating and lighting a three-story building fifty by sixty feet, spending one-third of our appropriation for stock and equipment and paying twelve people with an appropriation of $18,000 a year. Aside from the most rigid economy and the exaction of self-sacrifice on the part of the members of the staff, the laboratory has suffered extremely from the uncertainty of its existence. For ten years New York State has provided for this work in the supply bill. This bill never passes the Legislature until the last hours of the session, and the members of the staff who are dependent upon their salaries for their livelihood, never have known from year to year whether the laboratory would be in existence the following year, or whether they would have to seek employment elsewhere. This is a state of affairs which has been most demoralizing for the laboratory and has seriously hampered the work of the institution. To-day we can look back upon ten years of creditable work. We have reached a point where in another year it will be necessary to ask the State to put at our disposal the means to apply the modern discoveries in cancer research to human beings, and if the State is to follow up the advantage which it has gained by the expenditure already made, and is to reap the reward of the industry of its own employees in its own laboratory, it will have to support this institution for at least ten years more, and for this reason we feel that the disadvantage produced by the uncertainty and precariousness of our existence should be removed by providing for the maintenance of the laboratory in the regular appropriation bill.

Furthermore, we have reached a point where the scope of our work and our usefulness to the State is distinctly impaired for want of funds. The State should appropriate for the maintenance of the laboratory during the coming year not less than $25,000. This sum is absolutely necessary to enable us to undertake most promising fields of research, from which we are debarred by want of funds, and also to correct certain unfair discrepancies in the salary list of the institute.

Cancer removed from the population of the State of New York in the year 1907, 6,400, largely able-bodied inhabitants. This is an increase of 232 over the preceding year, and as we pointed out in our last annual report, cancer in the State of New York, in proportion to the population has increased during the last ten years nearly 25 per cent. Tuberculosis during the same period has decreased in proportion to the population nearly 5 per cent. We reiterate these facts simply to point out that the State of New York is suffering every year a heavy loss in useful citizens and that cancer research has to-day entered upon a period which gives hopeful promise for ultimate checking and control, as well as cure, of this disease, and that the State, having once entered upon this work, it is not only bad business policy but wasteful extravagance of human lives to fail to support to the fullest extent, an investigation which has progressed to so promising a stage.

In May of last year, on our initiative, there was formed an American Society for Cancer Research. This society contains all the prominent investigators in this country. It held its first meeting in New York in November last at the Cornell University Medical School and Rockefeller Institute. On the 15th of April it will hold its second meeting in Buffalo at the New York State Cancer Laboratory. At that time this laboratory will have completed the first decade of its work. In justice to the record which this institute has made during that time, we feel that the Legislature of the State of New York should place us upon a sound footing, to demonstrate to the scientists of this country that the State is not abandoning the vital policy which dictated the foundation of this institute and its maintenance for ten years.

The expenses of the laboratory for the past year have been as follows:

1906.			**1906.**		
Sept. 30.	**Balance . . . .**	**$2,748 13**	**Sept. 30.**	**Appropriation 1906–07 . . . . . .**	**$18,000 00**
1907.					
Sept. 30.	**Stock and material**	**2,077 88**	**1907.**		
	Equipment	**662 07**	**Sept. 30.**	**Balance . . . .**	**1,077 46**
	Expense	**2,261 72**			
	Salaries	**11,327 66**			
		$19,077 46			**$19,077 46**

The staff of the laboratory is practically unchanged from last year.

Owing to changing the period for the annual report from October to January, the scientific publications which fall within the year 1907 are fewer than usual, a number of publications having appeared earlier in the year and been included in the Seventh Annual Report.

I have again to express to you the appreciation of the staff of the laboratory for the interest, support and consideration which our work has received at your hands during the past year.

Very truly yours,

HARVEY R. GAYLORD,

Director

THE RHYTHMS OF GROWTH ENERGY IN MOUSE CANCER

By GARY N. CALKINS

In 1906 Bashford, Murray and Bowen* published a paper on the experimental analysis of the growth of cancer in which the attempt was made to ascertain the biological growth relations of cancer cells in the successive transplantations. Statistics of greater numbers of mice that had been inoculated with cancer were studied by them and the results were plotted in a curve in which ordinates represent the percentage of "takes" and the variable abscissas, the length of time required by the cancer cells to grow into a tumor fit for re-inoculation. This graphical method, although crude, showed unmistakable evidence of alternating high and low virulence, expressed by rhythmical fluctuations in the curve. The English observers maintained that the combined factors, percentage of takes and period of development, give a more or less accurate means of measuring the relative growth energy of cancer cells (the term growth energy being adopted as a substitute for the abused term virulence) and that this growth energy runs in rhythms of higher and lower potency in successive transplantations. Furthermore Oskar Hertwig and Poll† in 1907, studying the growth phenomena of cancer cells came to a similar conclusion and offered in evidence a number of curves showing similar rhythmical variations.

On first thought such rhythms of growth energy would be expected in rapidly and constantly dividing cells for such is the case in young embryonic cells and in free-living cells where the conditions for development are maintained. Thus for Paramecium protoplasm‡ which was kept under observation for twenty-three months and each cell division recorded, it was found that the division energy or growth energy, was manifested in regular cycles while in each cycle according to Woodruff's* later observations, there are definite rhythms of division energy. In these experiments food and environmental conditions were kept as nearly uniform as possible, and it was safely demonstrated that the rhythmic character of the division activity was due to some inherent factor within the living protoplasm. In regard to similar rhythms of growth in cleavage cells, Wilson† says, "During the cleavage the individual blastomeres are often found to exhibit entirely different rhythms of division, periods of active division being succeeded by long pauses, and sometimes by an entire cessation of division even at a very early period."

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* Bashford, Murray & Bowen. Proc. Roy. Soc. B. 1906. Vol. 78.

† Hertwig and Poll. Zur Biologie der Mäusetumoren. Abh. d. kön Preuss. Ak. d. Wiss. 1907.

‡ G. N. Calkins. Studies on the Life History of Protozoa. IV. Jour. Exp. Zool. I, 1904.

Rhythms of greater and less division activity seem, therefore, to be characteristic of living protoplasm when actively dividing and whatever the cause may be for the fluctuations, they appear to be perfectly normal growth phenomena.

But it is quite a different matter when we return to rhythms of so-called growth energy in cancer cells, for here, according to both the English and German observers mentioned, the rhythms occur in successive transplantations of the tumor material and involve the uncertain factors of infectiveness, natural immunity, change of soil, and the various conditions of the operations. To compare these rhythms of division energy the rate of growth in each individual tumor, and not in successive tumors should be ascertained, and this is apparently an impossible task. It occurred to me that Bashford, Murray and Bowen's use of the percentage of takes as an arbitrary index of growth energy when in conjunction with the period of growth, was inadequate and unfortunate in that it confuses the growth energy with another factor characteristic only of malignant tumors, viz., the power of infection. The growth energy or division energy is better indicated by the time factor alone for it involves no factor of infectivity while the drawbacks to its use, such as natural and variable resistance in different mice, are the same in both cases.

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* L. L. Woodruff. Studies in the Life History of Hypotrichous ciliates. Jour. Exp. Zool. 2, 1905.

† E. B. Wilson. The Cell in Inheritance and Development. 1901. 2d ed. p. 389.

In a paper now in press I have made a study of the records of transplantations in our Brooklyn tumor* and have pointed out that the curves representing the infectivity and the growth energy of the cancer cells were quite independent of one another after the cancer cells had become accustomed to transplantation. It was shown, furthermore, that the rhythms of "growth energy" as described by the English pathologists, were not apparent in the curve indicating the growth energy when the time element alone was considered, but that the rhythms are well marked in the curve representing the infectivity. Diagram 3 shows the relations of these two curves for the Brooklyn tumor while Plate XX gives the curve for the same tumor made according to the method adopted by Bashford, Murray and Bowen. The history of the transplantations on which these curves are based shows that the growth energy is quite independent of the infectivity. This is best shown in the block curves where the two factors are contrasted. To make this diagram the percentages of takes for all transplantations of the same generation of transplants, were averaged and charted, the successive averages giving the curve of infectivity (solid line). Similarly the numbers of days required by all the tumors of any generation of transplantation to kill the mice were averaged, the successive averages giving the curve of growth energy (broken line).

The most noticeable feature in these curves is that the first few generations of transplants show a clearly marked relationship between the two curves. As the line of infectivity, i. e., the percentage of takes, rises from eighteen to fifty-three, the line of division energy, i. e., the average number of days required to kill the mice, descends from 94 to 33. The usual method of expressing this fact is by the statement that as the number of days decreases the virulence of the cancer-cells or their infecting power increases, or vice versa, and this has been the experience of all observers. But when the curve of infectivity is once established, the variations of the growth energy have no effect upon it.

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* **G. N. Calkins. The So-called Rhythms of Growth Energy in Mouse Cancer. Jour. Exp. Med. X, 3, 1908.**

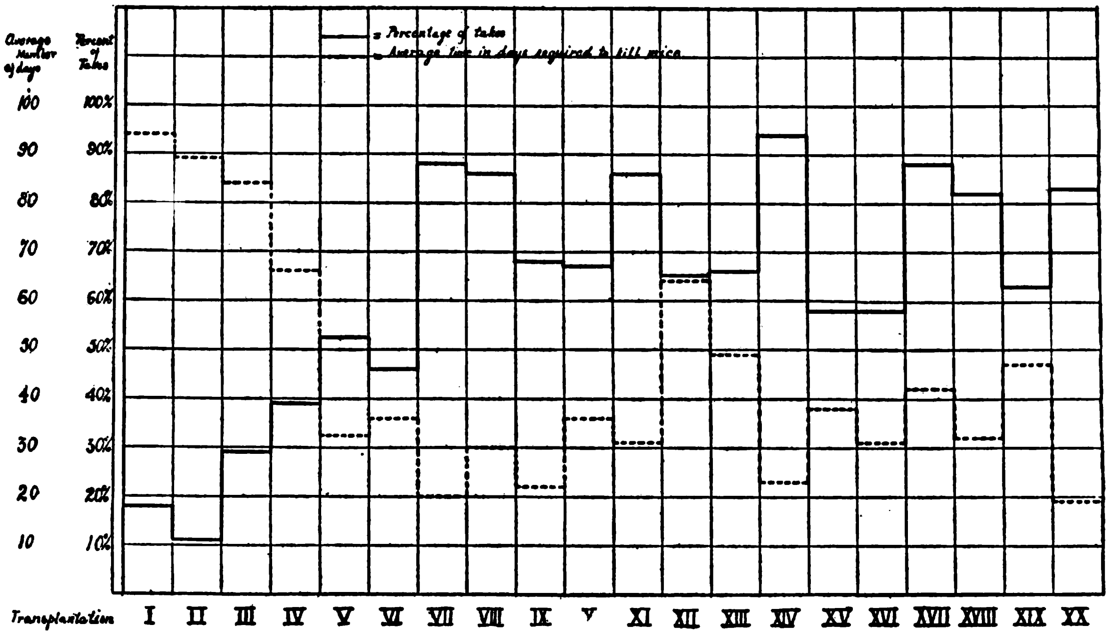

Average Number of days
Percent of Takes
= Percentage of takes
= Average time in days required to kill mice
100
90
80
70
60
50
40
30
20
10
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
Transplantation
I
II
III
IV
V
VI
VII
VIII
IX
V
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
XX

DIAGRAM III.

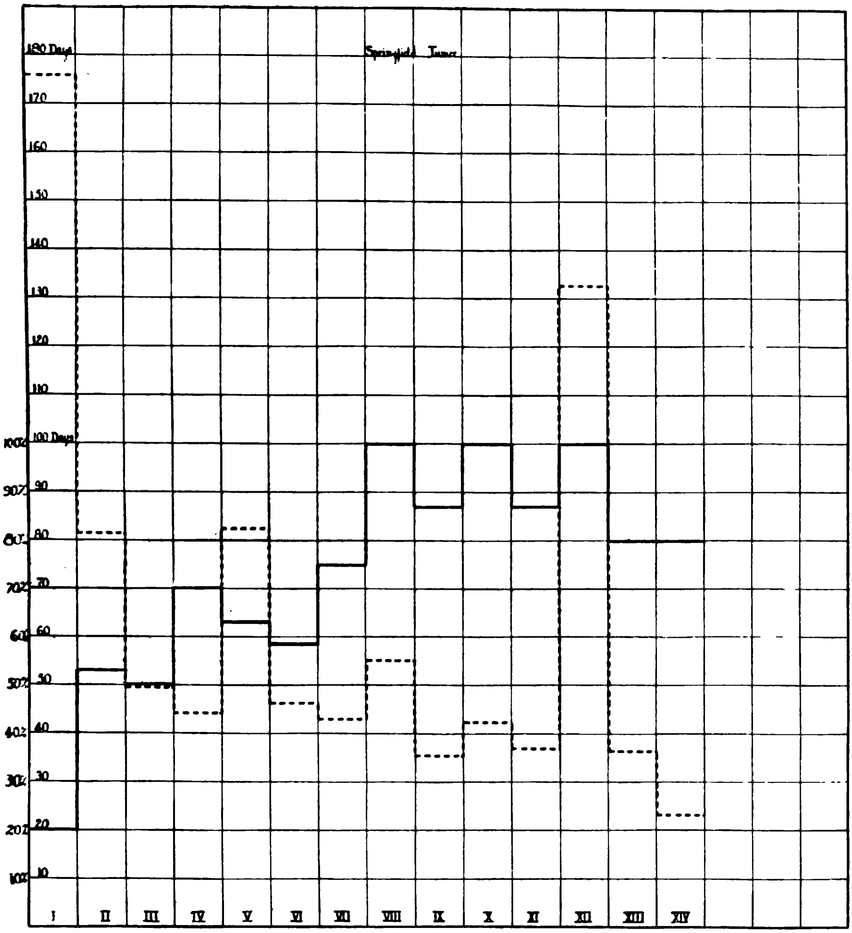

180 Days
Springfield Times
170
160
150
140
130
120
110
100%
100 Days
90%
90
80%
80
70%
70
60%
60
50%
50
40%
40
30%
30
20%
20
10%
10
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV

DIAGRAM IV.

This independence of the two factors is even more clearly shown in Diagram 4, representing the Springfield tumor, an adeno-carcinoma similar to the Brooklyn tumor. This is based upon the following statistics, the numbers of mice used being smaller than for the Brooklyn tumor.

GENERATION.	Total number of mice inoculated.	Dead in 10 days.	Total number tumors.	Killed for inoculation.	Dead from tumor.	Percentage of takes.	Average number of days.
I	*10	0	2	2	......	20	176
II	17	0	9	5	4	53	81.5
III	26	0	13	3	10	50	49.6
IV	26	1	18	3	15	70	44.2
V	33	3	19	5	14	63	†76.0
VI	53	3	31	2	29	58.5	43.7
VII	12	0	9	1	8	75	43.0
VIII	6	0	6	1	5	100	55.2
IX	10	2	7	2	5	87.5	35.5
X	15	1	14	1	13	100	42.4
XI	8	0	7	3	4	87.5	37.0
XII	3	0	3	2	1	100	‡132.6
XIII	12	2	8	2	6	80	36.6
XIV	17	2	12	2	10	80	23.4

* Ten additional mice were also inoculated with normal cancer mush *not* incubated. No tumors.

† Cancer mush not incubated.

‡ Tumor material kept in abdominal cavity of a rat for six days prior to inoculation.

Here again, at the outset, there is a definite relation between the two curves of infectivity and growth energy. The long period of development (176 days) accompanied the low percentage of takes (20 per cent.) characteristic of the first transplantation of a primary tumor. The second transplantation shows a well marked rise in infectivity and a corresponding increase in the rapidity of growth, the curve representing the latter falling from 176 to 81.5 days. From this period the rhythms in infectivity become manifest but do not become well marked until the latter half of the curve, when the percentage of takes is at a maximum. Contrasted with this rhythmical curve of infectivity is that representing the time factor or rapidity of growth of the cancer cells. Here there is no evidence of rhythmic rise and fall, but with two exceptional rises, there is a fairly constant descent in the curve indicating the gradual reduction of the time factor to what is possibly a minimum of twenty-three days.

The two exceptions are interesting and significant. The first one in the fifth transplantation, was probably due to the fact that for the first time, the cancer material was not incubated prior to inoculation. The second exception, in the twelfth transplantation, was much more noticeable, the time required for the tumor to develop increased from an average of thirty-seven days to an average of 132.6 days. In this case the tumor material before inoculation was put into the abdominal cavity of a rat for a period of six days, then taken out and treated as ordinary cancer material is treated for inoculation. One of the tumors from this lot that had required 211 days to develop was used for inoculation in the regular way, the result being a number of tumors which developed rapidly, the average number of days required to kill the mice being 36.6 days, or the same, practically as in the transplantation immediately preceding that with the material from the rat. There is every reason to believe that the effect upon the cancer cells of the sojourn in the rat was to reduce the rate of division or to decrease the growth energy, although we have no evidence as to how this effect was produced.

The most singular feature in regard to these two exceptional cases is that no corresponding variations were produced in the curve of infectivity. Every mouse inoculated with the cancer mush developed a tumor. Nor did the treatment affect the ordinary course of the rhythms. It follows, therefore, from these various observations that in this phenomenon which Bashford, Murray and Bowen, under the head of the growth energy, regarded as a single factor in tumor growth, we have at least two component factors which are independent of each other to a certain extent. The same percentage of takes is maintained after the tumor is established, regardless of whether they are fast or slow growing tumors.

A word as to the number of cases on which these various averages are based. The larger the number of units the more perfect any statistical computation must be, and the value of deductions from such curves depends upon their perfection in this regard. In cancer work, however, it is manifestly impossible to carry out experiments on a great numerical scale, and in practice only enough animals are used to ensure the perpetuation of the tumor.

This should be borne in mind in connection with any deduction that may be drawn from curves such as are here presented. For example, if a great number of mice had been used it is not probable that the percentage of takes would have reached 100, as given in the table and curve. Although the figures cannot be relied upon absolutely they may be sufficiently within the limits of error to give relative values. Thus the constancy with which the rhythms of infectivity occur in all strains of mouse tumors indicates a general biological phenomenon and not chance variations or variations within the limits of error. The conclusion that growth energy and infectivity are independent of one another, involving as it does a matter of considerable moment in cancer work, cannot be proved without much greater statistical evidence, but there is sufficient evidence, nevertheless, to indicate that such a conclusion is highly probable.

This conclusion is further borne out by a study of curves such as Bashford, Murray and Bowen presented. In these there is a certain advantage in being able to follow the vitality of cancer cells in different tumors of the same strain. Plate XX represents such a curve based upon the records of the Brooklyn tumor.

Transplantation of the primary tumor (1) was made May 30, 1905, the cancer mush being put into twenty-eight mice. None of these died inside of ten days, while five, or 18 per cent., developed tumors. One tumor suitable for transplantation developed in seventy-five days, while two of the cancer mice died. This tumor (2) was similarly teased and the cancer mush inoculated in the regular dosages, into thirty mice, of which three died inside of ten days and three, or 11 per cent., developed tumors. One of these tumors (*3a*) was ready for further inoculation in fifty-three days; a second (*3b*) and a third were ready in sixty-six days. These in turn were inoculated into different lots of mice of the fourth transplantation series, that lot inoculated with *3a* giving 33 per cent. of takes, and that from *3b* giving 25 per cent. The tumors from this second lot were used for further transplantations, the former for experiments and unsuitable for the present discussion. The mouse tumor designated *3b*, giving 25 per cent. of takes upon reinoculation, developed four tumors which were ready for transplantation in thirty-nine, fifty-one, fifty-three and sixty-four days, and they were inoculated in different batches of mice of the fourth series.

With the three transplantations which had been made up to this point the cancer cells had been growing from 180 to 200 days, and in this time there is a noticeable shortening of the period of development from seventy-five days after the first transplantation to thirty-nine days in the case of *4a,* and fifty-three days in *4b.* The increasing percentage of takes, combined with the shortening of the time of development, indicates that the cancer cells were becoming accustomed to the soil, to use Bashford's expressive term, of the different host mice, and were gaining strength with each new transplantation. That they were not yet perfectly adapted, however, is indicated by the fact that the tumors which developed upon the fourth transplantation were not equally established, some dying out altogether within two subsequent transplantations, while others gave rise to highly virulent cancers. Thus *4c* inoculated into a batch of mice produced only 18 per cent. of takes (*5b*), and these required forty-one days, while the same strain upon reinoculation into the sixth series, produced only 4 per cent., after which the strain died out entirely. Tumor *4b* had a more remarkable history for some of its cells were apparently endowed with a very high, and others with a very low, potential of vitality. This tumor, upon inoculation into the fifth series, produced only 15 per cent. of takes (*5f*), a marked decrease, but one of the tumors which developed in a short period of twenty-three days (*5f*), gave rise to a most malignant strain running up to 100 per cent. of takes in the twelfth transplantation (*12a*), while another tumor (*5c*) from the same source (*4b*) required sixty-four days and was comparatively feeble, for, upon reinoculation into the sixth series, it gave only 4 per cent. of takes. It is seen, therefore, that at this early period of transplantation, the same tumor may give rise to a highly virulent strain, or to a weak strain, showing that the cancer cells are not fully adapted to new soils. Of course there are always two factors to take into consideration, one is the division energy of the cancer cells, the other, the natural resistance of the host into which such cells are inoculated. It is not improbable that the batch of mice into which *4b* was transplanted had a relatively high protective reaction, so that the division energy of the cells as a whole was restrained, but the rapid growth of 5*f* shows that here was a mouse in which no such restraint was manifest, the natural resistance must have been weak or else the particular nutritive conditions must have been suitable, for the cancer cells acquired a high potential of activity. Tumor mouse 4*d* must have been similar to 5*f* in this respect, for the same cancer cells upon reinoculation into the fifth series, now gave 80 per cent. of takes (5*d, e*), as against 15 per cent., and in periods ranging from thirty-four to forty-two days.

The further history of the Brooklyn tumor is the working out of these two lines of development, one from 4*d,* the other from 5*f,* and in both strains the characteristic rise and fall in rhythmic change is well shown in the curve. The high percentage of takes from 4*d* is continued in the next transplantation, 5*d* alone being used as the others were taken for experiments. This tumor, upon inoculation in the sixth series, gave 77 per cent. of takes in the short periods of twenty-five (6*b* and *c*) and forty-three (6*a*) days. After this, however, the infectivity declined for these tumors upon inoculation into the seventh series, yielded only 60 per cent. (7*b*) and 33 per cent. (7*a*) in thirty-three and thirty-six days, respectively. The latter (7*a*) upon reinoculation, however, shot up again to 80 per cent. in the eighth series and in twenty-six days, thus marking a definite rhythm.

The history of tumor 5*f* is more interesting and is marked by a series of rapid growths and by a gradually increasing percentage of takes. It was transplanted into nine mice, of which two died in ten days, while in the others two, or 28 per cent., developed tumors. One of these died from the cancer; another (6*c*) had a transplantable tumor in seventeen days, and this, when transplanted into twenty mice, yielded 65 per cent. in twenty, thirty-nine and eighty days. In the latter case (7*d*) there is considerable evidence to show that the tumor was held back by the resistance of the mouse host, for the malignancy of the same tumor material was shown by the rapid growth of 7*c* and by the fact that 7*d,* when transplanted into a new batch, gave rise to 77 per cent. of takes and all within twenty-eight days (8*b, c*).

At this period in the history the curve becomes complicated through the massing of strains but they all show a certain optimum of development followed by a fall and this by a renewal of growth. The tumor 7*c* is a good average example; this, when transplanted into series eight gave 88 per cent. of takes, some (8*c* and 8*d*) in twenty-one days, one (8*b*) in fifteen days (8 ?) in twenty-six days. None of the mice inoculated died within ten days. The history of this set of tumors varies somewhat in the different cases; in some the divison energy of the cancer cells decreased immediately, in another it decreased only after the following transplantation. Following out the history of 8*c*, we see from the diagram that, upon reinoculation into a fresh batch of eleven mice, none died within ten days while seven, or 80 per cent., developed cancer, 9*d*, in thirteen days, 9*e*, in twenty-four, two others in thirty-eight, and two in forty-one days. Many of these were used for experiments, but two of the rapidly growing ones were transplanted (9*d* and 9*e*). In both cases there was a marked decrease in the division energy of the cancer cells: 9*d*, which had developed very rapidly (thirteen days), was transplanted into six mice of series 10, one of which died within ten days, while only two tumors, or 33 per cent., developed, these requiring twenty-five days. One of these two (10*e*), upon reinoculation into fresh mice of series 11, gave rise to 61 per cent. of tumors, two of which (11*f* and 11*g*) were used for experiments, the first developed in twenty-nine days, the second in seventy-seven.

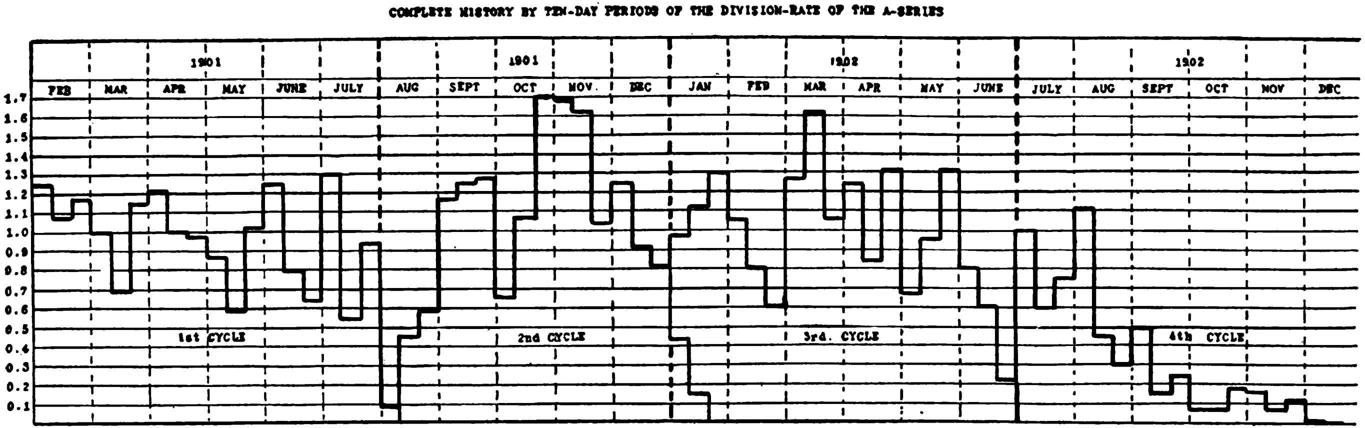
Turning back to 9*d*, which had the same ancestry as 9*e*, there is the same evidence of depression in the energy of the cancer cells coming from 8*c*. Tumor 9*d* was transplanted into sixteen mice, of which six died within ten days, while 66 per cent. developed tumors, of which the great number were used for experiments, all developing within thirty-one days. One (10*a*) was used for further transplanting into series 11; it was ready for inoculation in nineteen days, and was put into twenty-six mice of series 11. Of these four died within ten days, while ten, or 43 per cent., developed cancers, 11*c* in eighteen, 11*a* in thirty days. 11*a* was inoculated into ten mice, of which eight, or 80 per cent., developed tumors. Its history, therefore, is identical with that of 10*e* except that it was one generation behind the latter. There is the same lull in the division energy and the same recovery, and the same rhythm in infecting power.

All of the tumors described are to be traced back to tumor 8*c*, which came from the cancer cells in 7*c*. A very similar history is shown in the case of tumors coming from 8*b*, likewise derived from 7*c*. Here again the general result is a high percentage of takes with short periods of growth, or in general, a high virulence of the cancer cells. 8*b* was developed and ready for transplantation in fifteen days when it was put into twenty mice of the ninth series; none of these died within ten days, while eighteen, or 90 per cent., developed tumors, many being used for experiments of one kind or another, while four were transplanted into batches of the tenth series. The periods of development of the thirteen tumors were for the most part relatively short; 9*a* and 9 ? requiring only fourteen days, two others eighteen days, 9*b* and 9*c*, twenty days, three others, twenty-three, others, thirty, thirty-four and forty-four days, respectively. Of these 9*a*, 9*b* and 9*c* were used for further transplantation and their histories are remarkably similar. 9*a*, when inoculated into mice of the tenth series (10*d*), gave 75 per cent. of tumors which required from twenty-five to forty days to develop, and these tumors were used for experiments. 9*b*, when transplanted, gave a small percentage (43 per cent.) of takes (10*f*). There was, therefore, a well marked decline in the division energy of the cancer cells and the one tumor to develop required twenty-eight days and this, when transplanted into another batch of mice, caused their death inside of ten days. Of this ninth series, the tumor marked 9*c* was the most successful and also the most remarkable; it was transplanted into thirty mice of the tenth series, of which three died within ten days, while twenty, or 80 per cent., developed cancer in from fourteen to twenty-seven days, all rapidly growing tumors, two of which (10*b* and 10*c*) are given in the curve, the former developing in fourteen days, the latter in twenty. Each of these tumors, when transplanted into new lots of mice of the eleventh series, gave reduced percentages of takes. Tumor 10*b* fell to 62 per cent., 11*b* and 10*c* to 70 per cent. The progeny of 10*c* were used for experiments, while 10*b* was used for further transplantation. It was put into twenty-four mice of the eleventh series, of which none died, while fifteen, or 62 per cent., developed tumors as follows: 11*b* in seven (sic) days, another in twenty-four, 11*d* in twenty-six, others in twenty-seven, twenty-nine and thirty-two days, respectively. Of these tumors only 11*b* and 11*d* were used for further transplantation, the others going into experiments. Here, however, 11*b* is of especial interest, for upon inoculation into six mice of series twelve, 100 per cent. developed tumors (12*a*). Of these, one recovered spontaneously, while the others, when inoculated into new batches of mice, caused the death of all the latter within ten days (?). This malignant strain was thus brought to a speedy end.

The other strain from 10*b*, carried in mouse 11*d*, had a better fate. It was a more slowly growing tumor, requiring twenty-five days to develop. This tumor, upon transplantation in sixteen mice of series twelve, gave rise to 94 per cent. of takes in from twenty to twenty-eight days. Most of the resulting tumors were used for different purposes but one, 12*b*, which required twenty-five days, was again transplanted into thirty mice of series thirteen one of which died and 65 per cent. developed cancer in periods varying from twelve, fourteen, eighteen, twenty-three and thirty-one, to ninety-four days. Most of these were not followed up, one only, thirteen, which required thirty-one days, was transplanted into eight mice of the fourteenth series, giving 66 per cent. in long periods of development (fifty-five to sixty days).

Anyone who follows through these tedious details will see that the cancer cells contained in the tumor-bearing mouse, 7*c*, all had the same history of high proliferative energy followed by a decline. In all cases followed out, this decline is followed by a sharp rise in division energy and in the percentages of takes. Sister cells of tumor 7*c* formed the tumor in 7*d*, but required a much longer period (eighty days) to develop. Although slower, these tumor cells had a high potential of division energy, as shown by the fact of high percentages and quick periods in the eighth and ninth series. The tenth series, like the tenth series in the allied strain, brought a decline, thus marking for this strain a similar rhythm of growth energy.

The further history of the transplantations needs no special analysis; as the curve clearly shows, there is a continuation of the rhythms of infectivity, the average of which remains high, while the time element becomes much longer, until the curve now

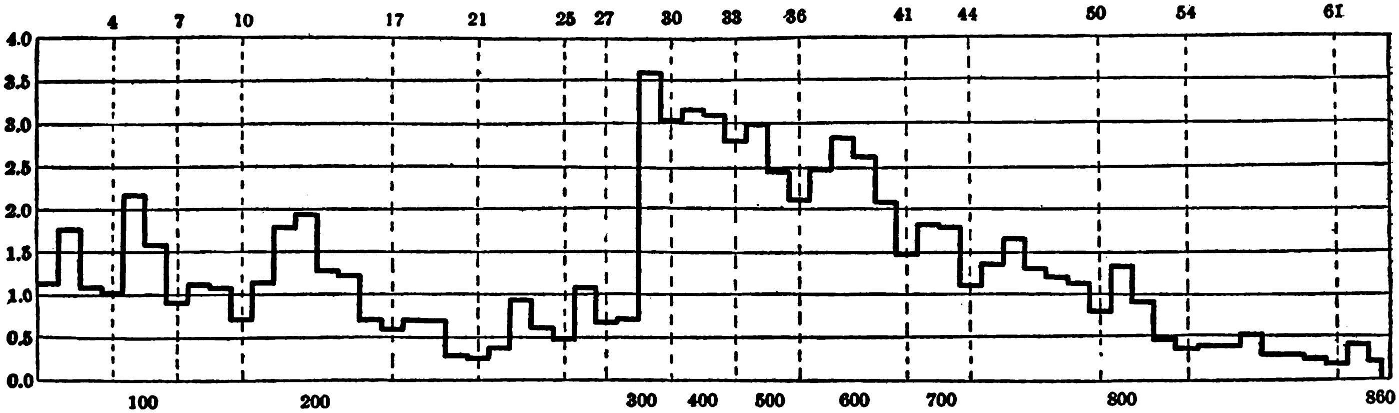


COMPLETE HISTORY BY TEN DAY PERIODS OF THE DIVISION OF THE A SERIES.— DIAGRAM I.

History of the A Series from start (Feb. 1, 1901) to finish (Dec. 19, 1902) by ten-day periods (three periods to each month).

The ordinates represent the average daily rate of division. The heavy dotted lines indicate the limits of the several cycles, and the lines of the curve carried to the base indicate that the individuals that were not stimulated by change of diet died out.

The points at which such lines leave the curve indicate the time of the successfully changed diet.



OXYTRICHA FALLAX, CULTURE A.

OXYTRICHA FALLAX, CULTURE A.— DIAGRAM II.

Complete history of Oxytricha fallax, Culture A, from start (October 26, 1901) to extinction (July 14, 1903) in the 860th generation. Rate of division averaged from ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. The broken lines designate the limits of the various *rhythms*. Above, the numbers of the ten-day periods which limit the rhythms are indicated; below, the monthsin which the rhythms chiefly fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained. (Woodruff.)

has the aspect of having been pulled out at the ends and concentrated in the middle.

It is hardly conceivable that this rhythmic development of the cancer cells is only artificial, due, that is, to the fact of inoculation into particularly susceptible mice, of which, according to Bashford, young mice offer the best examples. Such an explanation might be satisfactory for one or two instances of the phenomenon, but recurring regularly as it does, the phenomenon probably signifies something fundamental. In a developing embryo the rhythms of growth energy of the individual cells do not indicate a cessation of growth for the organism as a whole, for while some of the cells are resting others are dividing and developing. The energy of growth is applied sometimes in this sometimes in that group of cells. In cancer, however, there is only the one type of cells to develop, and appearing in different batches of mice the phenomenon cannot be attributed to the source of nutriment of the cancer cells, but must be looked for in the cells themselves. As previously stated these cells must be regarded as complete organisms, satisfying all of their physiological need through their own physiologically balanced activities. The source of the alternating rhythms must, therefore, be sought in the cells. In other and possibly analogous cases of complete cellular organism the cause of the alternating periods is looked for in the variation of the physiological activities. Thus in the *Paramecium* experiments already mentioned, high division energy of the individual cells is followed by a gradually lowering rate of division until it is reduced to nil. At such periods it was found that the cells could not reduce the highly stable condition of the protoplasm to a more labile condition, and that they evidently lacked the power of oxidation, or of forming the oxidative enzymes. This condition was relieved by the use of certain salts acting as stimuli; by them the labile condition was reëstablished and divisions in rhythmic waves of vigor again ensued (see Diagram 1).

In the *Paramecium* there was nothing to interrupt the dividing activity of the organism save the ordinary course of vitality. With the cancer cells there is always the struggle to maintain an existence against what must be adverse conditions. The division energy of the cells is retarded by such conditions, a result best shown in the early periods of the transplantations before the cells became adapted to the strange soil. The same phenomenon is manifest in the culture of *Paramecium,* a few days being required for the cells to become adapted to the culture medium. They seem to survive well enough in the medium, but they do not divide with such regularity during the first few days as afterward when well established. The difference is due to the density of the fluid medium in which the organism finds itself rather than to any change in food. And so it may be with the cancer cells; they have the same source of food in the new mouse to which they are transplanted, but the environment is a little different and it requires some time for them to get started. Once started, however, there seems to be but little change in their potential of infectivity, although the periods of development differ to some considerable extent, the difference probably being due to the protective reactions on the part of the host mice. This is shown well in Diagram 4 where the periods of development in days are contrasted with the percentages of takes in the different transplantations from start of the tumor to the present. The plotted curve shows that as the number of days required for development in the successive periods decreases, the percentage of takes increases with great regularity, until, when once established, it is maintained regardless apparently of the length of time required for development. This fact indicates that the cancer cells are endowed with a like power of infection, but that the host animals exert a retarding influence upon their development. All cancer cells cannot be expected to develop, hence it is too much to expect 100 per cent. of takes all the time; those cells maintain their existence which are endowed in the parent tumor with the most advantageous equipment for growth. This is not to be expected in imperfect cells, or in degenerated cells, but in those having the full complement of cell organs, normal number of chromosomes, division energy and the like. The success of such cells may be compared with that of the gametocytes of the malaria organisms; these are endowed with some property through which they are able to withstand the digestive fluids of the mosquito's gut, while all other forms of the malaria organisms are digested. So with the cancer cells those persist which have the proper attributes and these attributes are apparently developed with some degree of periodicity. Is there any evidence to indicate the nature of these particular attributes?

The cancer cell gets its nourishment as do other epithelial cells; there is little evidence to support the belief of Ehrlich that they make use of a specific *Nahrsubstanz,* although they are undoubtedly so modified as to take up more nourishment. They are bathed by the same fluids, they are subject to the same general conditions of metabolism as other epithelial cells. Other epithelial cells, however, do not multiply beyond the limits of organic regulation; these cells do and they are cancer cells because of this power. Other normal epithelial cells, beyond one or two divisions, do not multiply upon transplantation into other mice. The cancer problem, therefore, has to do with the specific attribute which distinguishes the cancer cells from these other epithelial cells, and we are again brought back to the question — what is the origin or source of the stimulus which causes this infectivity combined with the increase of the division energy? The conditions of the environment of cancer cells in new batches of mice cannot account for the stimulus to continued development in successive transplantations; if this were true the percentage of takes and rapidity of growth would be quite as extensive on the first transplantation as in the later ones, for this would be the anticipated result if the cancer cells have that vigor of growth which should accompany them if they were embryonic tissue cells, or cells recently stimulated by some initial stimulus, or cells recently fertilized. The cancer cells must carry with them the sources of their stimuli, and these stimuli must be the same, both in the primary tumor and in the continued development of that tumor's cells, for we cannot conceive that each new batch of mice into which the cancer cells are transplanted provide the same identical physiological conditions as those of the first mouse in which the cancer was a primary tumor. Furthermore we cannot conceive any reason why, if the proper physiological conditions were present, there should be rhythmic variations in their production in different sets of mice, hence the conclusion is forced that the source of the stimulus to development is within the cancer cell itself.

In the entire realm of biology there are few analogues to this condition of affairs. The favorite hypothesis of the medical profession — the Cohnheim theory of embryonic tissue — rests as the cause of cancer, cannot stand the biological test. Leaving out of consideration that entirely unexplained set of questions having to do with the position and conditions of such cells during the periods of growth and maturity, the cancer cells, after the tumor is started, have none of the attributes of embryonic cells. Mere power of proliferation is not a sufficient characteristic, while the fundamental characteristics of embryonic tissue cells are usually entirely ignored by the advocates of this theory. These conditions are somewhat more subtle than are the ordinary manifestations of vitality, but they are none the less potent. I refer to the properties which all embryonic cells have of differentiation combined with coördination, self-regulation and adaptation. Cancer cells have none of these; there is no evidence of differentiation although mountains of mouse tissue might have been grown from the cell progeny of that original Jensen mouse. The absence of any regulation and of all coördination and of adaptation to the needs of an organism as a whole which is the goal of all embryonic cells, is evidence against the hypothesis. All of these factors are characteristics of embryonic cells, and to the biologist they are the most important and the most characteristic. No theory of embryonic tissue satisfies the conditions of increased vitality and vigor of growth shown by this Jensen tumor, and we must look in other directions for its origin.

Nor can the tumor origin be traced to any detached cell, which, freed from its supporting and regulating membrana propria, starts off on an independent career of lawless development. This theory of Ribbert's postulates the metamorphosis of an ordinary epithelial cell into a parasite endowed with a potential of indefinite vitality and development. It does not account for the origin of the detaching force, nor for the origin of the stimulus to the latent division energy, much less does it account for the perpetuation of that stimulus. Here, too, lies the difficulty with the theory of chemical stimulation which Marchand has elaborated. There is no evidence that an initial stimulus can continue to act in this direction indefinitely, the impetus given soon wears off, and the gradually increasing vigor of a tumor like that of the Brooklyn mouse, is evidence quite strong enough to indicate that the stimulus, whatever it is, acquires strength rather than loses it.

The summation of the evidence, finally, shows that the cancer cell carries with it into each new mouse into which it is transplanted its own stimulus to division. Furthermore this stimulus cannot be an integral part of its own protoplasm, nor created by it as a product of its own metabolism; the very definition of a stimulus involves the idea of an external origin. We are thus driven back into the last resort of a biologist or pathologist, to the suggestion that *the cause of the division energy lies in the stimulating poison from some self-contained and ordinarily invisible micro-organism whose rhythmical variations in vitality may, possibly, account for the rhythmic variations in infectivity.*

In this connection we find one of the very few analogues of cancer cell growth, the formation of a vegetable gall where the latent division energy of the plant cells is stimulated to renewed activity by a poison secreted by an insect. As often pointed out, however, this is only a far-fetched analogy, for the stimulus comes to an end, and the division energy wears off. A more significant case is the formation of tumors on the roots of certain plants like the cabbage and its allies. Here, in club root, the parenchyma cells of the root are entered by a minute protozoan parasite. The organism multiplies in the cell protoplasm, which reacts to the stimulus of the parasite's presence, and divides. The division is repeated until great tumors are formed, parasite and protoplasm meantime living amicably together in symbiosis.

As all attempts to explain the cause of cancer must at the present time be of the nature of tentative or working hypotheses, the suggestion of an ultra-microscopic or unrecognized organism is not out of place, the value of an hypothesis being measured by the number of phenomena which it accounts for. The old arguments in favor of the parasitic theory of cancer, familiar to everyone conversant with the subject, apply in the present case. Cage infection, early noted by Borrel, observed by Giard, Lignières, Michaelis, in the Buffalo laboratory, etc., finds its interpretation in such an hypothesis. The infectivity of cancer cells, a phenomenon by which these cells differ from ordinary epithelial cells treated in the same way, is added evidence which finds a counterpart in the tumors of club-root type. Contrasted with these are tumors like vegetable galls, benign tumors and teratomata of man, in all of which there is a well-defined division energy of the cells involved, but no infectivity on the part of these cells. In club root and in cancer, division or growth energy is accompanied by clearly defined infectiveness. In club root infectivity is due to a contained parasite, and the growth energy is attributed to the stimulus from that parasite.

In cancer we have found no parasite confined exclusively to cancer tissue, but analysis of the growth energy and the phenomena of transplantation give evidence to show that infectivity and division energy of the cancer cells are not one and the same thing and that each has its own variations. The growth energy or division energy of the cancer cells, measured by the periods required for growth in successive batches of mice, affords no evidence of rhythmic development such as we find in embryonic cleavage cells or in free living organisms like the protozoa; there is, in all probability, such a rhythmic growth but it must take place in the individual mouse if at all. The infectivity, however, shows a regular rhythm which cannot be attributed to the reactions of mice in batches or lots of varying number, but this periodicity may be explained as the regular variations in vitality, similar to those of paramecium, oxytricha or tillina of a parasitic organism.

It is frequently noted that a strain of cancer cells giving a high percentage of takes (for example 11*b* of Plate XX) upon inoculation in fresh batches causes the death of all of the mice within a period of ten days. Such cases cannot be traced to the division energy of the cancer cells, but to the infectivity, and possibly to a parasite.

Many parasites have been assigned to the human cancer cells. Protozoa of all types have been held responsible by one or more investigators, but in no case have the claims been made good. The various morphological products of epithelial or blood cells that have been called parasites have served only to weaken the hypothesis of the parasite origin of cancer, which, with its power of indefinitely continued proliferation is a very different thing, biologically, from a benign tumor.

It is certainly conceivable that a parasite of cancer may be too minute to be seen with the technique at our disposal. At the present time we know a great deal about the yellow fever organism; we know the period of incubation it requires in the human blood; we know that it requires from twelve to fourteen days to develop in the body of a mosquito before the latter is able to transmit the disease; we know that the disease cannot be transmitted in any other way, and yet, knowing all of these things, the organism of yellow fever has never been seen. It will pass through the finest filters, and belongs, therefore, to a group which we must perforce, until they are actually seen, consider as ultra-microscopic organisms. Such parasites might be adapted to life in the epithelial cell as well as the organisms of club root are, and there in the protoplasm might easily be overlooked. It has been suggested that a species of *Spirochæta* is responsible for yellow fever, and spirochætes have actually been seen in the kidney of yellow fever victims. But they apparently do not exist as such in the blood or in the mosquito. We know nothing about the life history of the spirochætes as a group; if it is analogous to the life history of most protozoa we might well look for stages in which the organisms are of ultra-microscopic size.

We continually find spirochætes in mouse cancer and it is not improbable that we attach too little importance to them. We find furthermore a certain relation between the virulence of mouse cancer and the numbers of spiral organisms present; in highly virulent strains the tissues are infested with the parasites and it is not beyond reason to assume that the rapid death of batches of mice inoculated with virulent cancer cells is due to these spirochætes. Nor is it inconceivable that the parasites increase the susceptibility or prepare the soil, so to speak, in a new host for the growth of the cancer cells. On the other hand, the same or a very similar spirochæte is found in mice not infected with cancer, a fact which weakens any theory that the spirochæte is the direct cause of cancer, although the fact is not fatal to such a theory. A more serious objection is that no spirochætes are found in human cancer, but the objection might be met with the argument that spirochætes may have been present at the outset to prepare the soil and to provide the stimulus to the potential dividing energy.

SUMMARY

In conclusion we may summarize the above biological observations in a series of these as follows:

1. Cancer cells differ from other epithelial cells in respect to: (*a*) Size relations of nucleus and cell body; (*b*) power of indefinitely continued division.

2. Cancer cells differ from embryonic cells in absence of: (*a*) power of differentiation: (*b*) power of adaptation and coördination of parts to whole; (*c*) power of self-regulation and limit of growth.

3. The continued development of the cancer cells is subject to the following factors: (*a*) The inherent potential of division of the cancer cells. (*b*) The natural resistance of the inoculated animals. The latter factor is usually regarded as the index of malignancy of a tumor and is based upon the percentage of takes together with the period required to kill the mice. Our experiments, however, show that the percentage of takes is independent of the time factor, and indicate the presence of a third factor which may be described as (*c*) the potential of "infectivity" of the cancer cells.

4. The potential of infectivity of cancer cells is characterized by more or less regular rhythms; these must be distinguished from rhythms of growth energy of the cancer cells which in all probability occur within the individual mouse. Without the division energy of the cancer cell this infectivity is inoperative, hence it follows that the cause of the infectivity lies within the cancer cell or is constantly associated with it.

5. Cancer cells differ from epithelial cells by virtue of this potential of infectivity combined with that of division energy. There is no reason to doubt that the latter is due to the action of stimuli and not to the liberation of a restrained growth power of embryonic tissue. There is reason to doubt that an initial and discontinued stimulus is responsible for these attributes of

the cancer cells. Some types of benign tumors, and vegetable galls, may be due to the action of such initial stimuli, but in them there is no infectivity. Teratomata, due to embryonic cells, have a high power of differentiation combined with their division energy, but there is no infectivity. Infectivity distinguishes all cancerous growths from normal epithelium and from benign tumors or teratomata.

6. The rhythms of infectivity of cancer cells, erroneously regarded as rhythms of growth energy by Bashford, Murray and Bowen, appearing as they do in successive batches of mice which we may legitimately assume to have like powers of resistance, must have their cause in the cancer cells themselves. These cells, therefore, must be equivalent to parasites, or else parasites are contained within or associated with them.

7. Upon any other hypothesis it is difficult to conceive of cells creating a continual internal stimulus to their own growth energy.

8. Many lines of evidence point to the presence of some possible organism within the cancer cell; some organism which, acting as does *Plasmodiophora brassicæ* within vegetable cells, underlies the infectivity of cancer cells and provides the stimulus for their continued proliferation. Upon such an assumption the numerous cases of cage infection find their explanation.

9. The various inclusions of the cancer cell which have been described as organisms have been disproved; yet the analogy of club root and the many filter experiments show that the cause of infection may lie within the cancer cell. It is conceivable that, like yellow fever organisms, such an incitant may be in the protoplasm and beyond our powers with the microscope to locate.

10. The spirochætes which we have found in mouse cancer may have something to do with this infectivity of cancer cells. They may be useful in preparing the "soil" in new mouse hosts and making it susceptible to cell growth; or they may have intracellular stages in their life history which are too minute to be seen. The rhythms of infectivity, finally, may be an expression of the vitality of these spirochætes or of the hypothetical ultra-microscopical organisms accompanying cancer cells.

A SPIROCHETE IN PRIMARY AND TRANSPLANTED CARCINOMA OF THE BREAST IN MICE*

HARVEY R. GAYLORD, M.D.

In the early spring of 1905 the writer first observed in the epithelium of a retrograding transplanted tumor of the Jensen series, certain curious structures which were the subject of a publication by G. N. Calkins and G. H. A. Clowes under the title "Some Artefacts in Mouse Carcinoma."[1] The material in which the structures in question were first seen was hardened in an excess of mercury and contained, besides the structures about to be specified, curious deposits in crystalline form, both intra- and extra-cellular. The structures which attracted our attention at that time were very fine filaments of varying length, frequently possessed of clubbed ends, located in the protoplasm. They are illustrated by Figs. 5 and 6 of Plate 17, and by drawings by Calkins in Figs. 6, 7, 8, 9, and 10 of Plate 18.

The first impression which we had of these structures was that they must be some sort of parasitic form as yet unrecognized, but our inability to distinguish them from the deposits of mercury in the surrounding tissues, and in the nuclei and protoplasm of the cells of the tumor, led to the conclusion by Calkins and Clowes that they were artefacts, and the specimen was thereupon described and published.

So deeply impressed was the writer that certain of these forms must be parasitic that he never abandoned that point of view; and that Clowes and Calkins were also of the opinion that the bodies represented unusual structures impregnated with mercury is shown by the following sentence in their publication, on p. 559: "While these deposits are to be interpreted as artefacts, the fact must not be overlooked that something of an unusual nature is present in these cancer cells and tissues, upon which the salts of the fixing agent work in forming the deposits of various kinds."

Being deeply impressed with the possible significance of these curious filamentous structures in the vacuoles of the cells of the

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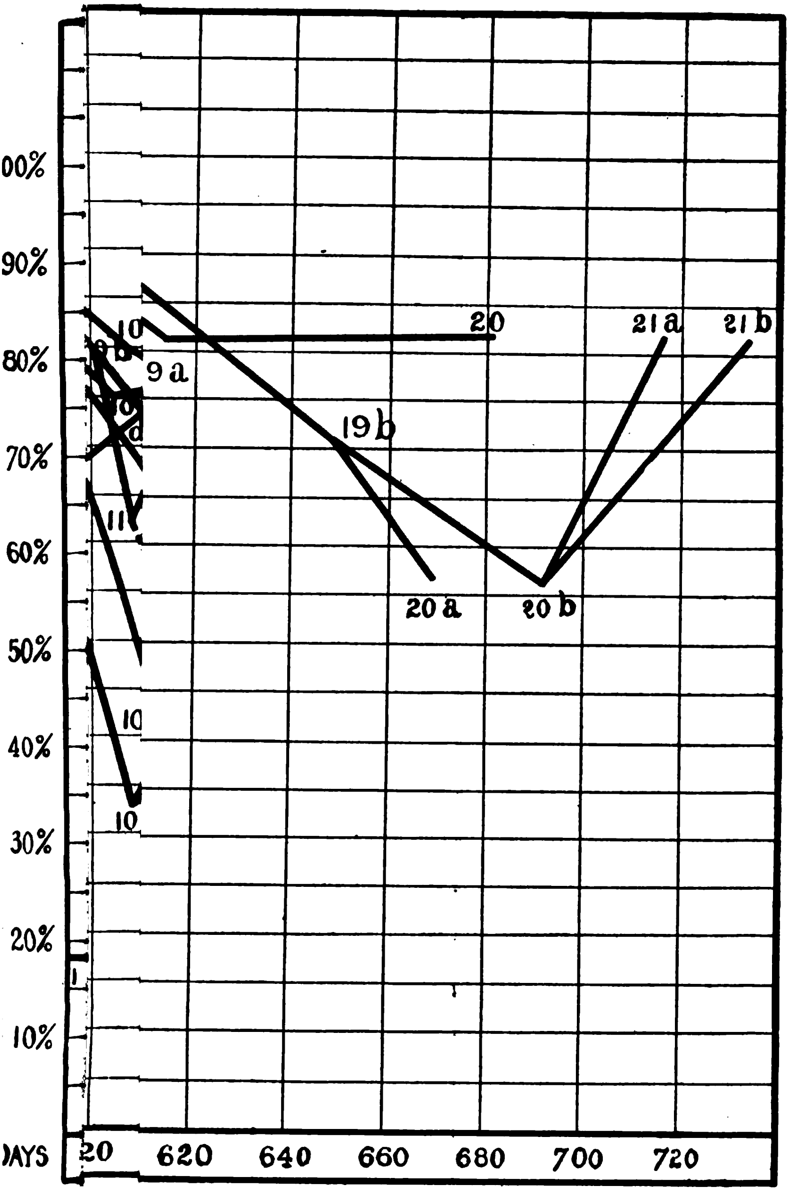
* Received for publication, March 12, 1907.

[1] *Jour. Infect. Dis.*, 1905, 2, p. 555.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transplanted mouse tumor, and having made various attempts to find a method which would better demonstrate them if present, the writer at once saw, on the publication of Levaditi's article,[1] a close resemblance between these filamentous structures and the involution forms of *Spirochæta pallida* so frequently encountered in syphilitic tissue. Thereupon a systematic investigation of all the transplanted mouse tumors in the State Cancer Laboratory was undertaken by the Levaditi method. The results of this systematic examination are here reported.*

An examination of transplanted tumors from three separate strains which are at present in existence in this laboratory has shown the constant occurrence of a characteristic organism in the transplanted tumors of each. The tumors from which these strains are derived are first the famous Jensen, which is now, in our hands, in its twentieth generation; a very virulent tumor known as the Brooklyn tumor, in its twentieth generation; and a less virulent tumor, known as the Springfield tumor, in its twelfth generation. All three of these tumors are carcinomata of the breast. They present practically identical characteristics, being carcinoma solidum, with occasionally adenomatous types.

The tumor known as 3^{10}, in which the original threadlike structures impregnated with mercury were discovered in March, 1905, belongs in the second generation of the Jensen strain. We have examined by the Levaditi method 914 E, 914 G, 915 A, and 915D, out of the fourteenth generation of this tumor. All of these transplanted tumors were movable beneath the skin and uncontaminated. At the margin of the tumors in the Levaditi sections is found in the immediate neighborhood of the infiltrating edge of epithelium, a small spiral organism from 2.5 to 7.8 μ in length, with from four to as high as 13 or 16 nodes or corkscrews. The organism is 0.6 u in average width; the ends are slightly rounded, and there is no evidence of an undulating membrane or flagellum.

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[1] *Ann. de l'Inst. Pasteur*, 1906, 20, p. 41.

* The title of this paper was on the program, but it was not read, at the meeting of the British Medical Association in Toronto, August 22, 1906. The facts in substance were presented at a meeting of the Academy of Medicine in Cleveland, January 11, 1907, and before the State Medical Society of New York at Albany, January 29, 1907.

Of the Springfield tumor 900 F, 921 F, 921 H, from the sixth generation, were subjected to the Levaditi method. An organism was found at the margin which was identical in appearance with that found in the Jensen tumors, bore the same relation to the epithelium, and was distributed in the same manner.

Of the Brooklyn tumor 741 A from the ninth generation, 763 E eleventh generation, 918 G thirteenth generation, 904 K thirteenth generation, 935 H fourteenth generation, 918 C fourteenth generation, 1039 E fifteenth generation, 1088 C sixteenth generation, 1113 A seventeenth generation, were examined by the Levaditi method. In every section of every one of these tumors the same characteristic spirochetes were found as described in the Jensen and Springfield tumors. These were in great numbers about the margin of the tumor in the connective tissue immediately adjacent to the epithelium. The organism is also occasionally found in the stroma or between the epithelial cells through the substance of the tumor, but the characteristic distribution is in the zone of round-celled infiltration and between the epithelial cells at the infiltrating edge of the tumor. Some of the larger of these tumors showed in their centers the usual areas of necrosis. A careful examination of these areas of necrosis failed to show the presence of any microörganisms of any kind.

Having determined the practically constant occurrence of this organism by the Levaditi method, attempts were made to stain it by Giemsa, by the various flagella stains, or by Wright's method, all without success, although the material which was used was known to contain large numbers of organisms. The organism could, however, be easily detected in fresh material. It is rather difficult to see because of its low refractive index and the high refractive index of the fluid portions of the tumor. Patient search has, however, in every instance in which fresh material has been examined, demonstrated well-defined, actively motile organisms. Measurements made of these fresh, living organisms correspond with those made of the hardened organism in the Levaditi sections. Dr. F. G. Novy, who examined for me a fresh preparation from an uncontaminated mouse tumor, made the following measurements: From 2.5 to 7.8 μ in length and from 4 to 13 turns.

The proper position of this organism and a more definite description of its characteristics have been kindly undertaken by Professor Calkins.

Having determined the constant occurrence of a living spirochete in all of our transplanted mouse tumors, it was of the greatest importance to discover with what frequency they could be found in unattached, uncontaminated primary mouse carcinomata. We have up to the present time examined ten primary mouse carcinomata. One of these was badly impregnated, so that it was impossible to determine the structure of the tumor satisfactorily, and it was therefore excluded. Of the remaining nine, the organisms have been found in all. They were found with no difficulty whatsoever in five. These are known as G-7, G-E, G-A, G-C, Springfield No. 8, and Buffalo I. In the tumors known as G-10 and G-8 the impregnation was imperfect and the organisms were of a pale brown, but could, however, be definitely distinguished. Tumor G-D was large and on its superior aspect slightly attached to the skin. The greater part of the central portion of the tumor was necrotic; only a very narrow margin at the periphery of the deeper portions of the tumor was in a state of active growth, and in these regions the organisms, although few in number, could be easily found.

It is to be noted that all of these tumors, with the exception of G-D and Buffalo I, were freely movable beneath the skin and small. All the G tumors were obtained at different times from a dealer in Massachusetts, whose animals are frequently affected with carcinoma of the breast. We have received from this dealer, in the course of the last eight months, thirty-one tumors. The tumor designated Springfield 8 was a small primary tumor obtained from a dealer in Springfield, Ohio, who has furnished us with three tumors in the last year and a half. It is from this dealer that we have obtained a cage from which in the course of three years have been taken over sixty mice with carcinoma of the breast. The cage with its contents was brought to Buffalo, and since its arrival at this laboratory five mice with cancer of the breast have been removed from it. Both of these series of tumor mice have been previously reported.[1] The Buffalo I tumor was found in a cage of pet mice in the home of a Buffalo physician. To these must be added a primary cancer of the breast found by Calkins in a mouse in his laboratory at Columbia University, New York, which contains the organism.

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[1] *Jour. Amer. Med. Assoc.*, 1907, 48, p. 15.

For control of the work up to this point we have impregnated and sectioned pieces from all the organs and subcutaneous tissues of five supposedly normal mice. A prolonged search of all these tissues has given negative results, but more extensive controls will be necessary before the distribution of this organism, which obviously must be very wide, can be defined.

Before passing to a study of the relation of these organisms to the structure of the tumors in which they occur, it may be well to review the scanty literature on the presence of spirochetes in mouse and other carcinomata.

The first reference to the presence of spirochetes in malignant tumors is found in the report of Hoffman.[5] Under Hoffman's direction Mulzer found spirochetes in the scrapings from a case of carcinoma of the cervix and in two squamous epitheliomata, one from the face and the other from the abdominal aspect. All of these tumors were *advanced and ulcerated.* In all three cases the spirochetes showed coarse gyrations and stained more deeply than *Sp. pallida.* Certain individuals were encountered, however, which did not stain so deeply, and in the size and number of their gyrations closely approximated the latter organism.

Löwenthal[6] also describes the detection of spirochetes by the Giemsa method, on the surface of ulcerated tumors. He calls attention to the fact that they are not only present in human tumors under such conditions, but that he has found them in numbers associated with the usual bacteria on the ulcerated surface of a tumor in the neck of a dog. He calls attention to the similarity of the organism he observed to a small spirochete found in feces.

This organism usually stains a pale blue with Giemsa, and stains also with a borax-methylene blue solution which does not stain *Sp. pallida.* The organisms are from 2.5 to 6 μ in length. He could not determine their thickness, but estimated it to be from 0.25 to 0.5 μ. They appear plumper when stained with Löffler's flagella stain. The gyrations are very close together and abrupt. The organism has usually from 4 to 12 corkscrews, and he estimates these to be about 0.5 μ apart. He proposes as the name for this organism "*Spirochaeta microgyrata.*" Up to the present he has been unable to determine the presence of flagella, and an undulating membrane was not visible, but he inferred from the increased plumpness of the organism, when stained with Löffler's flagella stain, that one was probably present. The long examples of the organism were frequently found to consist of two individuals attached at the ends. He considers that it may be readily differentiated from *Sp. pallida.* Besides this organism he frequently found a larger form from 5 to 11 μ in length with corkscrews from 1.5 to 2 μ apart which stains blue with

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[5] *Berl. klin. Wohnschr.*, 1905, 42, p. 880.

[6] *Ibid.*, 1906, 43, p. 283.

Giemsa. In all cases where Löwenthal detected the spirochetes on the ulcerated surface of tumors he found regularly rodlike, straight or slightly bent, sausage-like structures which in their appearance and form closely resembled the so-called fusiform bacillus which accompanies the spirochetes, known as *Spirillum sputigenum*, so frequently found in the buccal cavity. Several authors have already advanced the view that there is a genetic relation between these fusiform structures and the spirochetes with which they are associated. Löwenthal advocates the same view in connection with the fusiform structures accompanying the small spirochetes found on the surface of ulcerating tumors, for the reason that they invariably accompany these organisms. He states that wherever he found fusiform structures in a smear, on further search he never failed to find the small spirochetes in question.

Borrell[1] found helminthia in two inclosed mouse tumors surrounded by large numbers of leucocytes and endothelial phagocytes. The worms were transported to the tumors through the blood vessels after penetrating the intestinal wall. In the neighborhood of these worms Borrel states that large numbers of spirochetes were present, and in a very cachectic mouse sent from Ehrlich's laboratory, in which the tumor was not ulcerated, he found large numbers of spirochetes. These had coarse gyrations. In the two cases described in Paris the spirochetes were of different form, very small, and with closely packed spirals. The tumors thus examined were movable beneath the skin, were not ulcerated, and at the time of publication were still living. He concluded that it was impossible to draw etiological conclusions from these observations, but he found it of great interest that spirochetes and worms were found in these particular strains.

Spirochetes in unulcerated human cancer, demonstrated by the silver method, have as yet been reported by but one observer, Friedenthal.[2] Friedenthal is of the opinion that the structures, which are clearly spirochetes from his illustrations are not organisms, but represent condensations of the protoplasm impregnated with silver. He obviously published his observations to show that so-called spirochetes of syphilis which Schultze and others hold to be nerve fibres and elastic fibres impregnated by the silver method, are not necessarily all attributable to the misinterpretation. Inasmuch as the structures he described were within the epithelial cells of the tumor, Friedenthal held that some of them were condensations of the protoplasm, etc., and not nerve endings or elastic fibres.

It will be seen that of these observers Mulzer describes a coarse spirochete on the ulcerated surface of human tumors; Löwenthal describes a coarse and a smaller organism, known as *Sp. microgyrata*, on the surface of ulcerated human tumor and in a tumor in the neck of a dog. None of these authors attributes any significance to the presence of these organisms, as they were found under conditions which could not justify any suggestion that they were other than accidental in their occurrence. Borrel found in two cases a small spirochete in connection with helminthia and an organism with coarse gyrations in a cachectic mouse tumor sent from Ehrlich's laboratory. Borrel does not state whether these tumors were primary or transplanted tumors, but the inference is that they were transplanted. He believed that the organisms were conveyed to the tumors by the helminthia in the first two cases, and drew no conclusions as to their possible etiological significance in any of the three cases described.

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[1] *Comp. rend. de la Soc. de Biol.*, 1905, 58, p. 770.

[2] *Berl. klin. Wchnschr.*, 1906, 43, p. 283.

Besides spirochetes associated with mouse tumors, Wenyon[1] describes a spirochete which he found in the blood of a gray mouse in the Pasteur Institute. From this mouse he succeeded in infecting other mice; in these both the blood and the spleen contained the organism and could be used for further inoculation. He inoculated as many as fifty mice and never encountered a case of natural immunity. The organisms stained readily with any of the ordinary stains — Giemsa, fuchsin, methylene blue, etc.

"In stained preparations the spirochete is seen as a uniformly staining spiral; the longer forms, however, show a clear unstained central spot (see Diagrams Nos. 8 and 9). The ends are slightly tapering; there is no sign of a nucleus or undulating membrane. As just mentioned, the larger forms have a clear spot at their center, and in some of these the body of the spiral tapers toward this spot. In some cases two small spirals are attached end to end by an unstained region. These forms are evidently stages of transverse division. No indications of longitudinal division were seen nor any mode of reproduction other than the one just mentioned.

"The number of the turns of the spiral varies from six in the longest forms to two in the shortest. The lengths of the spirochetes vary from 6 or 7 μ to 3 or 4 μ. The width is about 2 μ.

"The spirochete was always seen in the spiral form; no other forms were found at any time."

Wenyon states that, after discussing the matter with M. Borrel, they arrived at the conclusion that the organism just described and the one observed by Borrel[2] in the juice of malignant growths of mice are the same organism; comparison of Borrel's preparations and those of Wenyon lead to the same conclusion. Wenyon attempted to test the theory of Borrel that these organisms had migrated into the tumors from the intestinal tract, by inoculating mice with mucus from the intestinal tract of mice which contained large numbers of varying kinds of spirochetes. These conclusions were negative; no infection or abscess resulted. The mucus from the intestinal tract of a mouse infected with *Spirochaeta m.* likewise gave negative results. Wenyon concluded that there is no evidence that these spirochetes have originated in the intestine.

The description given by Wenyon of the organism found in the blood of otherwise normal mice clearly distinguishes it from the organism which we have described. If it be identical with the organism found by Borrel in his mouse tumors that would seem to distinguish this organism from the organism which we have found. Borrel does not give the method by which he succeeded in demonstrating the organism found in his mouse tumors, but as his publication antedates that of Levaditi, it is highly probable that it was by means of the Giemsa or some aniline staining method. Whether our inability to stain our organism with any of the aniline methods will prove a means of distinguishing it from other small organisms, such as that of Löwenthal and the smaller organisms found by Borrel in combination with helmin-

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[1] *Jour. Hyg.*, 1906, 6, p. 580.

[2] *Compt. rend. de la Soc. de Biol.*, 1905, 58, p. 770

thia, remains to be seen, but the forms with the coarser gyrations appear to be clearly distinguishable from our organism.

The distribution of the organisms through the primary tumors examined is of great importance. A careful study of these tumors will appear to throw some light on whether or not the organisms were accidental in their relation to the tumors in which they are found, or whether they may bear some etiological relation. A description of one of these tumors will suffice for all, the only difference being in the number of organisms which the small fragments impregnated with silver happen to contain. The greatest number were found in the primary tumor known as G-7, and, with the qualification that the other tumors contain fewer organisms, the description of this tumor will apply to the remaining eight.

This tumor was sent from Massachusetts and remained in the laboratory about two days. The tumor was about the size of a small bean, measuring approximately 1 cm. by 0.6 cm. in its greatest diameter. It was slightly flattened, freely movable beneath the skin, and was situated on the ventral aspect. The mouse was killed and the tumor removed September 20, 1906. Weight of mouse 33.5 grams, weight of tumor 1.5 grams. Six mice, Lot 1031, were inoculated with portions of the tumor. Results to date negative. Small fragments of the tumor were hardened in formalin, and some of them impregnated by the Levaditi method.

An examination of a section of the tumor hardened in formalin and stained with Borrel's method, with low power, shows the tumor to be a rapidly growing adenocarcinoma of the breast. In many portions the tumor assumes the characteristics of a rapidly growing carcinoma solidum. At the edge of the tumor is the usual characteristic proliferation of the connective tissue invaded by prolongations and nests of epithelium from the tumor mass. In some portions of the periphery a more or less definite connective tissue capsule is present. Scattered through the tumor are the small areas of necrosis which characterize rapidly growing tumors of this type. The stroma is in some portions of the tumor poorly developed, in others well defined. Here and there, especially at the margins where the tumor is penetrating into the connective tissue structure, the tendency to the adenomatous type is quite marked. In these regions cysts of considerable size can be found, filled with the usual coagulated fluid mixed with cell detritus. A careful examination of such a section with oil immersion fails to show the presence of any organisms, even after comparison with sections from the immediate neighborhood impregnated with the Levaditi method in which plentiful spirochetes are present. Not even unstained structures which one might identify can be determined.

Sections from small pieces of the tumor impregnated by the Levaditi method, examined under high power, present a very striking appearance. First of all, lying free within the cysts in the coagulated contents, are found sharply defined, intensely black spirals, measuring from 2.5 to 7.8 μ in length. The corkscrews are from three or four to 13 or even more in number. These organisms are more or less regularly distributed through the cyst spaces (see Fig. 4, Plate 3), and present so characteristic an appearance that they can, to our minds, never be confused with nerve endings. In the rapidly growing portions of the tumor between the epithelial cells one frequently encounters aggregations of five or six, or even a dozen, of these characteristic organisms lying in small, clear spaces between the epithelial cells, as though they were surrounded by some clear fluid producing a form of vacuole (Fig. 6, Plate 3).

Passing to the margins of the tumor, one finds that in the nests of epithelium penetrating into the connective tissue stroma the lumena of the small tubular structures frequently contain aggregations of organisms, and here and there free epithelial cells with organisms in their protoplasm. One can easily imagine that the development of the larger cysts in the tumor might be determined by the proliferation of the epithelium around the small groups of organisms thus found. Beginning with a secretion of fluid around the organisms, as shown in Fig. 6, Plate 3, the proliferation of the epithelium might produce the beginning of a cyst, such as is represented by Figs. 8 and 3; and the continued secretion of fluid and associated proliferation might ultimately terminate in the development of a larger cyst, such as Fig. 4. The invariable presence of organisms in the larger cyst cavities would suggest such a possibility. The organisms are distributed in this tumor in such a way that the largest numbers of organisms are in the most rapidly growing portions of the periphery. Occasional organisms are found in the connective tissue beyond the growing edge of the tumor. This is especially well seen where the epithelium is penetrating into the adjacent fat tissue. Here organisms will be found betwen the fat cells a short distance in advance of the epithelium, but when one investigates the surrounding normal tissues beyond this zone, no organisms are to be found. Where the stroma of the tumor is best developed one frequently finds organisms in the stroma. Where this is not so well defined, they lie betwen the epithelial cells, as shown in Figs. 3, 6, and 8, Plate 3. Occasionally organisms can be found within the epithelial cells. Here they are frequently curved into ring forms (Fig. 7, Plate 3), or in aggregations suggesting agglutination (Fig. 7, Plate 3). The characteristic spiral form of the organism is not always apparent. Occasional forms will be met where the silver appears to have impregnated the organisms homogeneously. This is usually the case with organisms within the protoplasm of epithelial cells (Figs. 7 and 8, Plate 3), and in these cases the organism frequently presents a beaded appearance suggestive of involution forms or degeneration approaching disintegration. In regions where phagocytosis is active one will find fragments of the organisms in the protoplasm of the epithelium, the organisms in these cases appearing to break up into minute granules or short S-shaped structures similar to the changes in *Sp. Obermeieri* in mononuclear macrophages in the peritoneal cavities of hyperimmunized rats (Pfeiffer's phenomenon).

The examination of sections of the remaining eight carcinomata of the breast in mice differ in no way from the findings in G-7. In the tumors in which there are few organisms these are found exclusively at the very margin of the growing edge, in the center of cell nests, and in the connective tissue stroma surrounding the tumor.

In the transplanted tumors the distribution is even more pronouncedly at the margin of the tumors. In rapidly growing, early transplants, the organisms are massed in the zone of connective tissue proliferation at the margin, and in the more virulent tumors, such as the Brooklyn tumor, the organisms are frequently present in great numbers (Fig. 5, Plate 1). Involution forms and disintegrating organisms are very frequent in these areas. Phagocytosis does not seem to be so active in these tumors. Careful examination of the sections from the Springfield, Jensen, and Brooklyn strains shows no differences in appearance or measurements, distribution, or characteristics of the organisms in the different strains. We appear here to be dealing with a definite organism. In large, late transplanted tumors, in which there are areas of extensive necrosis, the organisms are exclusively confined to the growing margins of the tumor. When, as occasionally happens, the necrosis extends to the periphery of the tumor, the adjacent connective tissue zone contains either no organisms or occasionally disintegrating or involution forms. In two large tumors examined, which were badly contaminated with bacteria, no spirochetes whatever could be found.

With the determination of the practically constant association of this characteristic organism in primary and transplanted mouse tumors naturally arises the question as to their significance. Although it will require a prolonged and careful search through other types of tumors, especially in other animals and in human beings, before the relation of this organism to the tumors in question can be definitely ascertained, still it would seem not improper to discuss in the light of the striking evidence of cage infection in this very group of tumors, the possible etiological significance of these organisms. Up to the present time those who have sought for parasites in cancer have searched for an intracellular organism, and at first glance it would seem difficult to understand how an organism with the distribution of the one in question could be responsible for the epithelial proliferation necessary for the production of a malignant growth. We have for some time assumed that extracellular organisms might be the cause of the proliferation of epithelium through the medium of some toxic substance which they elaborate. Evidence of the existence of some bio-chemical substance of this sort in tumors has come to us in the course of our experimentation. The interesting work of Clowes and Baeslack from this laboratory on "The Influence Exerted on the Virulence of Carcinoma in Mice by Subjecting the Tumor Material to Incubation Previous to Inoculation," is a case in point, Clowes having reasoned that the best explanation of the marked increase in virulence produced by a short period of incubation resulted from the effect of increased temperature upon the rate of reaction of some stimulating substance contained in the tumors. These interesting observations have had a very positive and new light shed upon them by the work of Bernhard Fischer.[1] Fischer has made the discovery that there are certain chemical substances (thus far the fat stains, scarlet R, Sudan III, and indophenol) which possess a positive chemotactic quality for the epithelium of the skin in rabbits, and possibly some other animals. This chemotactic or attractive quality is exerted only when these fat stains, dissolved in olive oil, are injected into the subcutaneous tissue beneath the skin. Here they set up a chronic connective tissue proliferation similar to that found about the margins of beginning epitheliomata of the skin. The scarlet R oil penetrates into all the lymph spaces and crevices of the tissue and, with the advent of the chronic connective tissue proliferation, exerts a chemotactic attraction for the epithelium of the deeper layers of the adjacent skin. Oil alone does not exert an activity to this extent, and it is Fischer's opinion that part of the scarlet R, although but slightly soluble in water, is taken up by the lymph and transplanted to the epithelium which it stimulates to proliferation. Within a short period (three weeks) the presence of the scarlet R in the subcutaneous tissue produces an active proliferation of the epithelium of the skin, of the hair follicles and sebaceous glands, associated with the presence of typical and atypical karyokinetic figures. The epithelium penetrates into the surrounding connective tissue in the form of characteristic prolongations and nests, such as characterize the beginning of squamous epithelioma of the skin. Typical epithelial pearls are formed, and Fischer states that at this stage of the process the histological appearance is indistinguishable from epithelioma of the skin. The illustrations which he gives confirm this opinion. That it is the scarlet R which attracts the epithelium is shown by the epithelium growing down to, surrounding, and gradually removing the saturated oil drops. Where the scarlet oil penetrates into the lymph spaces the epithelium proliferates in the lymph spaces in pursuit of it, and in one case, where Fischer wounded the cartilage with the needle used for injection, he found the epithelium penetrating into the lymph spaces of the cartilage and the clefts which contained the scarlet oil. That part of the coloring matter is diffused through the tissues; and that it is in this way that scarlet R produces its first effect upon the more or less distant epithelium is shown by the occasional extensive staining of the fat constituents of the adjacent cartilage cells where the scarlet R has been injected into the connective tissue in the immediate neighborhood. Fischer found that scarlet R affects only the epithelium of the epidermis of the rabbit. Attempts to produce similar proliferations by injecting the stain into the breast, under the epithelium of the stomach and intestinal tract, have proven invariably negative. In one case of a dog in which a large amount of scarlet R was injected beneath the skin producing the characteristic proliferation of the epithelium, small nodules apparently derived from proliferation of the alveolar epithelium of the lung were found, and Fischer suggests that possibly this type of epithelium may prove less specific than others and also react to the stimulus of scarlet R.

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[1] Münch med. Wchnschr., 1906, 53, p. 2041.

From his experiments it would appear that scarlet R is capable of affecting only one kind of epithelium, the epidermis. Upon the cells of this structure it exerts, from its position in the subcutaneous tissue, an attractive or chemotactic function which causes the epithelium to proliferate in the deeper structures, producing in the height of its activity, a picture indistinguishable from beginning carcinoma. Here, however, the analogy ceases; for when the scarlet R is entirely absorbed by the epithelium, the cells rapidly hornify, and the entire process subsides. Fischer points out that all that is needed to extend this process into carcinoma would be the local, continuous production of some chemical substance similar in its affinities and characteristics to scarlet R. In a footnote he states that he appreciates that the advocates of the parasitic theory can utilize his observations in support of some parasite working through the medium of an *attraxine,* as he calls this substance; in which case it would be necessary, however, to assume a special organism for every type of epithelium subject to cancerous transformation.

The distribution of the organism which we have described corresponds in a very striking degree to the distribution of the scarlet R in Fischer's experiments. The absorption and removal of the scarlet oil by the epithelium finds its counterpart in the evidence of phagocytosis which we have noted. If the spiral organisms found in our tumors are the cause of these tumors, then they produce the proliferation of the epithelium through the medium of some toxic substance which they elaborate. That such a toxic substance possibly exists has, as we have pointed out, already been shown by Clowes. To our minds, the necessity of assuming a different organism for each type of epithelium rather simplifies than complicates the problem, and it is not impossible that organisms belonging in the same class, or of widely different characteristics, may possess the power of elaborating specific toxic substances. A striking possibility in this connection is the so-called Bilharzia disease, in which typical cancer of the bladder is associated with the presence of the embryos of the worm in the bladder wall. It is also of interest that in Bilharzia disease no metastases have ever been found, although the local disease of the bladder presents the characteristics of infiltrating carcinoma.

The evidence of immunity associated with these tumors is, to our minds, likewise suggestive of a possible etiological significance of these organisms, and, in this connection, the conditions found in the tumor 3^{10} referred to at the beginning of this article are of great interest. This tumor at the time of its removal and hardening had begun to retrograde and presented the histological characteristics of spontaneously retrograding tumors. It is possible that this tumor was hardened at the moment when a very active phagocytosis on the part of the remaining epithelium was in progress. As shown in Fig. 1, Plate 3 of this article, and in Figs. 5 and 6, Plate 17 of Calkins and Clowes's article in this journal, many of the epithelial cells of this tumor contained large numbers of small, rodlike structures, which, in the writer's opinion, are the organisms we have described, incrusted with mercury. If one studies the vacuoles containing these structures in sections of this tumor, one can trace through the smaller vacuoles a gradual disintegration and final disappearance of these structures within the vacuoles. The whole presents a picture which strongly suggests the description given by Novy and Knapp[1] of the disintegration of *Sp. Obermeieri* in the bodies of phagocytes after the injection of blood containing large numbers of this organism into the peritoneal cavity of recovered rats. As the entire process in the case of *Sp. Obermeieri* occurred in a period of less than ten minutes, it would appear that, if the appearance found in this tumor illustrated such a phenomenon in the mouse tumors, the tumor was placed in the hardening agent at the psychological moment. It will, however, require further experimentation to confirm the significance of these appearances.

It is obviously too early to draw far-reaching conclusions from the presence of this organism in our mouse tumors. First, of all, more extensive experiments must be carried out. These are in progress, and the organism is being studied in all its relations to the tumor in question, in which investigations Drs. Calkins and Clowes have joined. The description of what appear to be similar organisms impregnated with silver in a case of uncontaminated human cancer of the breast by Friedenthal naturally suggests that a further search for these organisms in human tumors may lead to positive results in this connection, and such a systematic search has already been inaugurated. At present it appears to us that the constancy with which this organism is found in the primary and transplanted mouse tumors is very striking; that in the light of Fischer's work it would appear to us quite possible that an extracellular organism distributed as this organism is distributed, could be the cause of the proliferation; that the evidence of phagocytosis and the detection of these organisms at times within the protoplasm of epithelial cells would sufficiently explain the establishment of metastases by the transportation of infected cells. The organism should be found with continued regularity, in which case it would appear to bear the same relation to these tumors as does *Sp. pallida* to syphilis.

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 291.

SUMMARY

1. In 1905 there were found in vacuoles in the epithelial cells of a retrograding mouse tumor, fine, rodlike structures impregnated with mercury. These the writer held to be parasites.
2. Attempts to fix these structures were not successful until the advent of Levaditi's method. With this method a characteristic spiral organism 2.5 to 7.8 μ in length and 0.6 μ in width, with four to thirteen turns per individual, has been demonstrated in ten consecutive spontaneous carcinomata of the breast in mice obtained from Massahcusetts, Ohio, and New York. In one other tumor the hardening method was a failure.
3. An examination of sixteen transplanted mouse tumors from three different sources shows the presence of the same organism in all tumors examined.
4. An examination of fresh materials from all transplanted uncontaminated tumors from these strains demonstrates the organism in the living state. It is frequently motile and is found with difficulty.
5. Measurements made on the fresh organism correspond closely with the measurements made in the stained preparations.
6. The distribution of the organism through the primary tumors shows that they are most prevalent in the most actively growing portions of the tumor; that they live in the connective tissue at the margin of the tumors, and in the stroma of the tumor; that they are found between the epithelial cells of the tumor and in the cyst cavities of the tumors where these are present.
7. In the early transplanted tumors the organisms are found in the connective tissue zone at the growing edge and between the cells at the growing edge.

8. The more virulent tumors contain the greatest number of organisms.

9. In both primary and transplanted mouse tumors evidences of phagocytosis on the part of the epithelial cells are to be found. The organism in these cells frequently assumes the form of rings and breaks down into small S-shaped segments and granules.

10. In two tumors badly contaminated by bacteria no spirochetes could be found.

11. The examination of organs and subcutaneous tissues of five normal mice by the Levaditi method has shown no spirochetes.

12. All attempts to stain the organism with aniline stains to date have proven unsuccessful.

13. The organism is morphologically distinguished from the organism described by Wenyon in the blood of mice.

14. It would appear to be distinguishable from the *Spirochaeta microgyrata* of Löwenthal and the organisms found by Borrel in mouse tumors, both of which are stained with the Giemsa stain, by its inability to take this stain.

15. Our observations do not as yet establish an etiological relation between this organism and cancer of the breast in mice, but the presence of the organism in primary mouse cancers with which it is regularly transplanted through many generations, greatly increasing in number as the tumors increase in virulence, instead of interfering with, and finally preventing, transplantation as do bacteria, is suggestive.

DESCRIPTION OF PLATE 1

FIG. 1.— Epithelial cell with large vacuole in protoplasm. Nucleus pushed to one side. Vacuole filled with fine rodlike bodies some of which show gyrations and beadlike structure. Taken from Jensen mouse tumor 1905. Sublimate fixation. Organism unstained. ×1,030.

FIG. 2.— Spirochetes from margin of transplanted Springfield tumor. Carcinoma solidum of the breast. Levaditi silver method. ×1,360.

FIG. 3.— Spirochetes between the epithelial cells of primary adenocarcinoma of the breast in mouse G-7. ×1,030.

FIG. 4.— Section through cyst in carcinoma of the breast, mouse G-7, showing three spirochetes in cyst contents. ×1,030.

FIG. 5.— Large numbers of spirochetes in margin of rapidly growing transplanted carcinoma of the breast, Brooklyn tumor. ×1,030.

FIG. 6.— Group of organisms between the epithelial cells of primary carcinoma, mouse G-7. ×1,030.

FIG. 7.— Spirochetes in protoplasm of epithelial cell, same tumor as Figs. 3, 4, and 6, showing phagocytic action of epithelium. Spirochetes in form of a ring and agglutinated organisms. ×1,030.

FIG. 8— Spirochetes in protoplasm of epithelial cell at the center of nest of growing epithelium. ×1,030.

In Figs. 1, 3, 4, 5, 7, and 8 the spirals are not easily seen, owing to the low magnification which was chosen to show the relation of the organisms to the tissue. In Figs. 2 and 6 the spirals are perfectly distinct. In every case, however, except in Figs. 7 and 8 the spirals are plainly discernible through the microscope. In Figs. 7 and 8, representing degeneration forms, the spirals are not intact.

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PLATE 1.

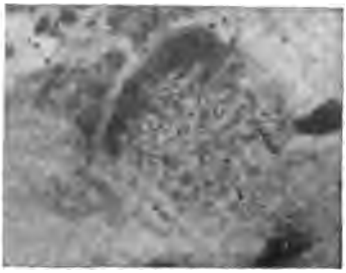

FIG. 1.

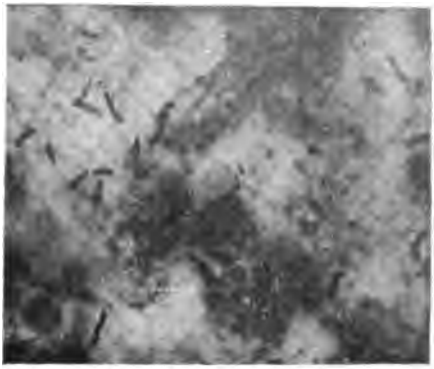

FIG. 2.

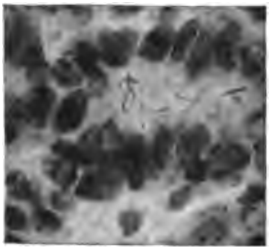

FIG. 3.

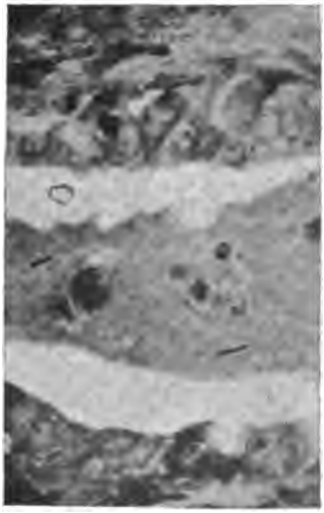

FIG. 4.

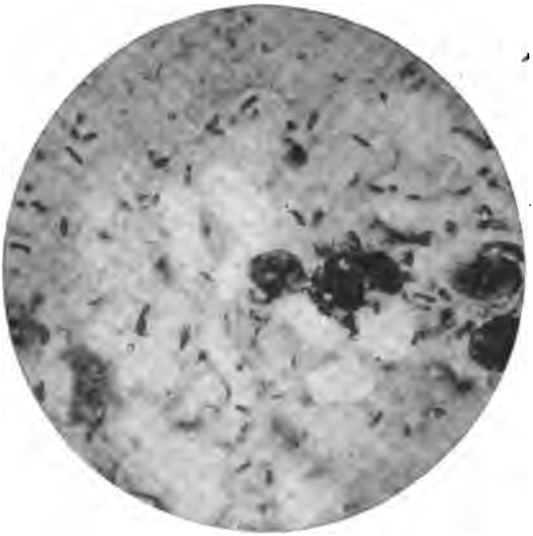

FIG. 5.

FIG. 6.

FIG. 7.

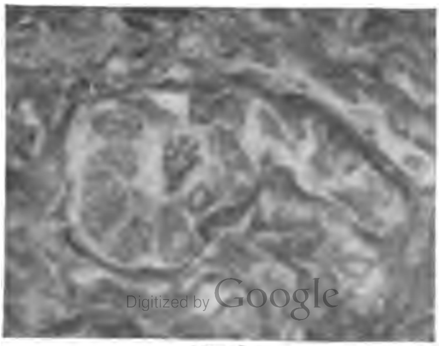

FIG. 8.

A SPIROCHETE IN MOUSE CANCER, SPIROCHAETA MICROGYRATA (LOWENTHAL) VAR. GAYLORDI *

BY GARY N. CALKINS

WITH the uncertainty at the present time which attaches to species of *Spirochaeta,* it does not seem wise to make a new one for this parasite of the mouse tumors. Both in the case of *Trypanosoma* and of *Spirochaeta* new species have been created on the basis of purely physiological grounds, and many of the supposed "good" species of both of these genera will probably turn out to be mere variants of one or another. The mere fact than an organism will not live in the blood which serves as a culture medium for another organism of apparently identical structure and character is not enough to satisfy the requirements of a new species. There are many species of protozoa which are represented by salt-and fresh-water forms, and although the fresh-water forms cannot live if placed in salt water, nor salt-water forms in fresh water, no one would think of making different species of these organisms because of their mode of life. So it is with these spirochetes and similar flagellates; a physiological difference, while important in matters of therapeutics, has little value in taxonomy, and the creation of new species therefore. may well be left until the life-history of different forms is made out.

Thus it is with the spirochete in mouse cancer which Gaylord discovered last summer. In dimensions and in general character the organism agrees perfectly with the one which Löwenthal described in 1906 in ulcerated human carcinoma, in a dog tumor, and in feces; but his spirochete stained readily with the Giemsa stain, and the present one stains with the greatest difficulty. It will be remembered that this difference was the sole distinction between *Sp. refringens* and *Sp. pallida* at the time of their discovery, although subsequent observations have shown that the one has an undulating membrane and no flagella, while the other has flagella and no membrane. In view of this difference in staining capacity and in the host of the organism on the one side, and

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* Received for publication, March 11, 1907.

for convenience in reference in future work on the other, I venture to give to this parasite in mouse cancer a new variety name, with the understanding that should future research show it to be a distinct species, the variety name shall become the specific name. Löwenthal's species name *Spirochaeta microgyrata* is sufficiently distinctive to characterize the organism, and to this I would add the variety name *Gaylordi,* in recognition of the fact that Dr. Gaylord was the first to study the relation of this organism to these tumors.

With the exception of Löwenthal's original description,[1] there has apparently been no subsequent recognition of this species. We may have seen it in 1905 as the basis of the curious artefacts which appeared in one of the mouse tumors at the Buffalo laboratory, but it was certainly not recognized at that time as a spirochete. Borrel[2] may have seen it in 1905 in the three mouse tumors in which two forms of spirochete were observed, but no measurements and no figures were given, and no importance was attached to the presence of the organisms. Wenyon[3] evidently did not see it in his *Spirochaeta muris,* for the dimensions which he gives belong to an altogether different organism.

There is considerable question as to what constitutes the "unit" organism in *Spirochaeta,* and the majority of specific characterizations in consequence are vague and misleading. Most observers give the number of "turns," or, as I shall call them, "nodes" (a node representing the part from the crest of one undulation to the crest of the next adjacent), and the total length, both of them varying within rather wide limits. Such wide variations, especially when the organisms are supposed by many to divide by longitudinal division, are incompatible with what we know of unicellular forms generally, and the a priori conclusion is that these diverse sizes are the result of end-to-end union of smaller units forming variable aggregates which recall other catenoid colonies among the protozoa. Löwenthal, Krzysztalowicz and Siedlecki, Schütz, and others describe the breaking-up of spirals of *Sp. pallida* into smaller elements which are variously interpreted, while Novy describes the fragmentation of *Sp. Obermeieri* under the action of phagocytes. These observations afford some evidence for the view that the unit *Spirochaeta* is much smaller than the forms ordinarily seen.

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[1] *Berl. klin. Wchnschr.*, 1906, 43, p. 283.

[2] *Compt. rend. de la Soc. de Biol.*, 1905, 58, p. 770.

[3] *Jour. Hyg.*, 1906, 6, p. 580.

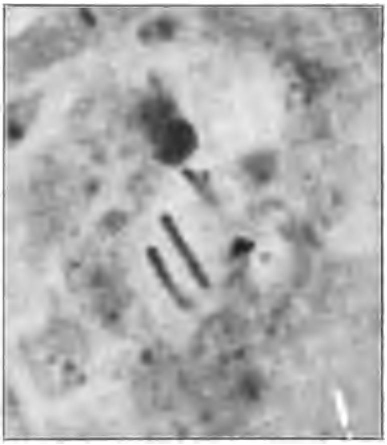

FIG. 2.— A group of spirochetes (two in focus) from a primary tumor from the zoological laboratory, Columbia University. ×2,500.

At the present time, and until the life-history is known, the most satisfactory basis for identification of the spirochete of mouse cancer is the measurement of the single node. This may be determined either by direct measurement of the node or by ascertaining the total length of the organism and the number of nodes, and thus getting the average size of the node. As it ordinarily appears, the organism measures from 2.5 μ. to 7.8 μ. in length, and it is made up of from 4 to 13 nodes. The average length of the node in a great many measurements is 0.6 u, and the organism is 0.6 u wide. The nodes therefore are characteristically crowded and the undulations steep. Compared with *Sp. Obermeieri, Sp. refringens,* and *Sp. pallida,* this form is much shorter, has more crowded nodes and a greater diameter both relatively and absolutely (Fig. 1).

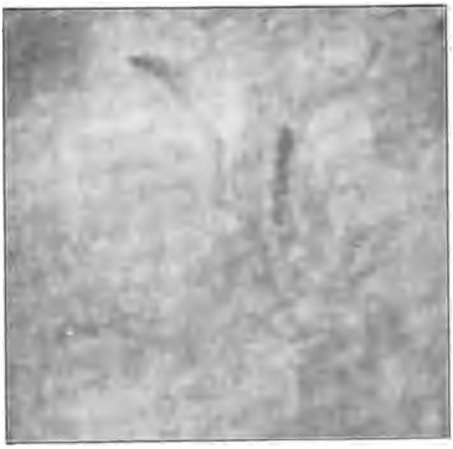

FIG. 1.— *Spirocheta microgyrata* Löw., var. *Gaylordi*. A single specimen from a transplanted tumor of the Jensen series. ×2,500.

The ends of *Spirochaeta microgyrata Gaylordi* are blunt and rounded and there is no evidence of tapering at the extremities, nor of undulating membrane, nor of flagella. (The apparent flagellum seen in Fig. 1 is an illusion caused by a slight fold in the membrane of the host cell in which the spirochete lies.)

In this preliminary communication it will not be possible to describe all of the changes which the spirochete undergoes in the cancer cells. Some of the changes that have been observed are undoubtedly phases in development of the organism as ordinarily seen, while others are undoubtedly involution forms. These different conditions are best seen in primary tumors, several of which have been obtained at the Buffalo laboratory and one at Columbia University in a mouse that had not been near the Buffalo laboratory, nor had it been near mice or cages from that place. I state this in some detail because of the possible criticism that spirochetes of this same type are found in all the tumors at the Buffalo laboratory because of a widespread infection there. Fig. 2 is a photograph of spirochetes from the primary tumor that developed in the Columbia laboratory, and Fig. 3 is a photograph from the same tumor showing a reduced condition of the spirochetes, perhaps due to the phagocytic action of the cancer cells.

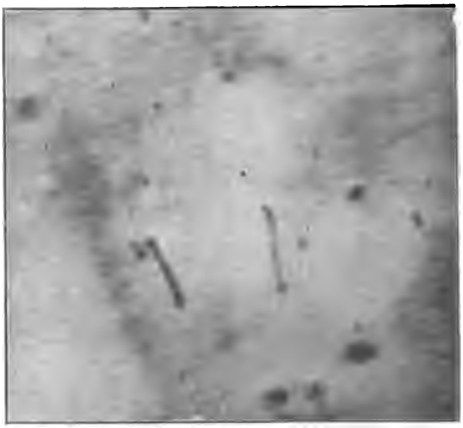

FIG. 3.— Two individuals undergoing degeneration. From the Columbia tumor. ×2,500.

Various enigmatical structures are found in the nuclei of the epithelial cells of these primary cancers — structures which I have failed to find in any of the normal glandular tissue which has been treated in the same way as the cancer tissue (i. e., by the Levaditi silver impregnation method). For this comparison

I have examined carefully tissue from thyroid, liver, intestine, and, in addition, mesentery connective tissue and muscles, all from normal mice, and in no case have I found the organism or intranuclear bodies mentioned above. In a future publication I hope to be able to throw some light on the significance of these intranuclear structures.

ABSTRACT OF THE WORK DONE IN THE NEW YORK STATE CANCER LABORATORY DURING 1907*

Dr H. R. Gaylord reported from the New York State Cancer Laboratory further observations on a breeding establishment in Massachusetts where cancer in mice is endemic. This is the same establishment which was reported at a meeting of the British Medical Association in Toronto in August, 1906. At the present time the laboratory has records covering eighteen months, during which time it has received fifty-one tumors in mice and knows of twelve other tumors in mice supplied to other scientists during this period, sixty-three tumors in mice developing during the period of eighteen months. In this time this breeder has reared approximately 7,000 mice. These fifty-one tumors, with the exception of one, have occurred in female mice. Forty-nine of them are tumors originating in the breast in females, one a squamous-celled epithelioma of the vulva in a female and one a fibro-sarcoma surrounding the right humerus. The breast tumors were distributed over the abdominal aspect and corresponding to the normal distribution of mammary tissue in the mouse. They presented everything from a simple adenoma to carcinoma solidum. In practically every tumor transition from simple adenoma to adenocarcinoma and carcinoma solidum could be traced. Fifteen of these tumors were transplanted; three took in the first generation and died out in the second; one continued and is now in the sixth generation.

Within the last year this breeder has begun breeding white rats at our suggestion and in stock coming from her establishment, we have found four rats with spontaneous tumors. All of these tumors have been fibro-adenomata of the breast, with the exception of one which is unquestionably an adenocarcinoma. During this period she has raised about 1,000 white rats.

The laboratory has had business relations with a breeder in Washington for the past three years, who sells about 2,500 white mice per annum and as many white rats. We have had a standing reward with him for mice or rats with tumors. He has never been able to supply us with a single tumor from his own stock but as evidence that he thoroughly understands the significance of our reward, he has secured a tumor mouse for us from another dealer.

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*Reported at the first meeting of the Association for Cancer Research. November 15, 1907, by Dr. H. R. Gaylord.

The laboratory possesses at present five transplantable mouse carcinomata and the spindle-celled sarcoma of the thyroid in a rat previously described as developing, with two other cases of fibrosarcoma, in a cage which had been used by Dr. Leo Loeb for sarcoma rats brought from Chicago. This sarcoma of the thyroid has now been transplanted through eight generations. The number of successful inoculations was surprisingly high in the first generation, giving five tumors in twenty-three inoculated rats, =21.8%. In the second generation but six tumors developed in 172 inoculated rats, being 3.5%; the third generation fourteen tumors in sixty-one rats =23%; fourth generation eight tumors in 125 rats =6.4%; fifth generation twenty-nine tumors in ninety rats =32.2%; sixth generation thirty-seven tumors in 260 rats =14.2%; seventh generation twelve tumors in 123 rats =9.8%; eighth generation ninety-nine rats have been inoculated, in which thus far but four tumors have developed.

Summary.—Total number of rats, 854; total number of tumors, 111; percentage of tumors, 13.

These tumors have frequently reached great size, even exceeding that of the rat. As will be observed from the figures, there is no notable increase in virulence, the relatively high percentages obtained in one generation frequently falling low in the next. This is probably owing to the lack of susceptibility in certain races of rats. The tumor appears to take just as well in parti-colored rats as in white rats. There have been occasional spontaneous recoveries and these rats have proved immune. The histological characteristics of the tumor have not changed; it presents the characteristics of spindle-celled sarcoma.

A careful examination has been made of the different strains of transplantable mouse tumors in the laboratory in search of sarcomatous changes in the stroma of transplanted mouse carcinomata, as first described by Ehrlich and Apolant and recently observed by Bashford. We have not thus far any evidence of such transformation in any of our transplanted strains.

A few months ago the laboratory came into possession of a female thoroughbred English bulldog suffering from round-celled sarcoma of the vagina of the type described by Smith and Washbourn, Sticker, Bashford, Ewing and Beebe. Through the kindness of Dr. Beebe, a spontaneously recovered and supposedly immune dog was sent to us from New York and Dr. Crile kindly came to Buffalo and after deep bleeding transfused the blood of the immune dog into the dog with the primary round-celled sarcoma. During the first two weeks there was marked diminution in the size of the tumors, the mass which entirely filled the perineum decreased in both dimensions two centimeters and then remained stationary for a period, and in our opinion was beginning to grow, when a second partial transfusion of the blood of a normal dog was performed. This made no impression on the tumor. A third transfusion was undertaken, employing two immune dogs furnished by Dr. Beebe, but unfortunately the bulldog was lost through the anæsthetic. From this experiment it would appear that round-celled sarcoma acquired in the natural way by dogs, may be influenced by transfusion with the blood of dogs rendered experimentally immune by spontaneous recovery from an inoculation tumor.

We wish to call attention to an apparently new phenomenon observed in the State laboratory in the sarcoma rats. Dr. Flexner has stated that there is no difficulty in implanting a second tumor in an animal in which a first inoculation has been successful, as opposed to the observation of Ehrlich, later confirmed by Clowes, that in mice the establishment of a first inoculation tumor usually prevents the development of a second inoculation. In attempting to repeat this observation in our sarcoma rats, animals were reinoculated every six days, with the result that it has been found that some twenty-five rats out of forty thus inoculated have developed more than one tumor, which have persisted side by side for a period equal to that usually required to kill when the tumors were not inhibited, and then retrograded. The tumors made their appearance successively, some growing as large as small hazelnuts and then remaining stationary for a period of from twenty to thirty days, when the tumors entirely disappear in a period of from seven to eight days. In only one case did a rat develop two tumors which grew until exitus. A number of these rats have been reinoculated, some of them a year later, and have proved to be immune. This phenomenon has occurred so often that it appears to be characteristic of the present state of virulence of this tumor and suggests that repeated successful inoculations may, under certain conditions, tend to bring about immunity, where one inoculation does not suffice. It is possible that the marked difference in this respect between our tumor and Dr. Flexner's in which multiple grafts regularly grew side by side to exitus, may be explained by the great virulence (90 to 100 per cent.) of his tumor, and the relatively low virulence (13 per cent.) of ours, but it appears that in many respects each tumor is a law unto itself, with the exception of certain basic phenomena which have now been found with sufficient regularity to establish their character as fundamental.

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